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INDEX OF SUBJECTS.

	Page
Aeration, Observations on the Influence of, on the Nutrient Solution in Water Culture Experiments—Stiles, Walter, and Jörgensen, Ingvar	181
Algæ (Myxophyceæ, Peridineæ, Bacillarieæ, Chlorophyceæ) by G. S. West, Cambridge Botanical Text Books, Vol. 1 [Review]—F. E. F(ritsch)	48
Bacillarieæ, <i>see</i> Algæ.	
Baeyer, <i>see under</i> Jörgensen, Ingvar, and Stiles, Walter.	
Branching in the Filicales, Observations on the Evolution of (1 Text-fig.)—Sahni, Birbal	1
Cambridge Botanical Text Books, Vol. I. Algæ (Myxophyceæ, Peridineæ, Bacillarieæ, Chlorophyceæ), by G. S. West [Review]—F. E. F(ritsch)	48
Carbon Assimilation, <i>see under</i> Jörgensen, Ingvar, and Stiles, Walter [Index of Authors].	
Cereal Crops, The Effect of Weeds upon—Winifred E. Brenchley	53
Chlorophyceæ, <i>see</i> Algæ.	
Classification of the Compositæ, History of the, <i>see under</i> Small, James [Index of Authors].	
Compositæ, The Origin and Development of—James Small	157, 198
Cordaitales, <i>see under</i> Fossil Plants.	
Cycadofilices, <i>see under</i> Fossil Plants.	
Cycadophyta, <i>see under</i> Fossil Plants.	
Endotrophic Mycorrhiza of Ericaceæ, The (4 Text-figs.)—Jean Dufrénoy	222
Energy Relations in Carbon Assimilation, <i>see under</i> Jörgensen, Ingvar, and Stiles, Walter (Index of Authors).	
Ericaceæ, The Endotrophic Mycorrhiza of (4 Text-figs.)—Jean Dufrénoy	222
Evolution of Branching in the Filicales, Observations on the (1 Text-fig.)—Birbal Sahni	1
Filicales, Observations on the Evolution of Branching in the (1 Text-fig.)—Birbal Sahni	1
Flowers, The Syrphid Visitors to certain—E. and H. Drabble	105
Fossil Plants, Vol. III (Pteridospermeæ, Cycadofilices, Cordiates, Cycadophyta), by A. C. Seward [Review]—D. H. S(cott)	230

	page
Hart, John William (E. N. T.)	45
Holden, Ruth (A. C. S.)	154
IN MEMORIAM—	
Ruth Holden (1890-1917) [A. C. S(eward)]	154
John William Hart [E. N. T(homas)]	45
<i>Leptosphaeria acuta</i> , On the Forcible Discharge of Spores of (15 Text-figs.)—W. J. Hodgetts	139
Mycorrhiza of Ericaceæ, The Endotrophic (4 Text-figs.)— Jean Dufrénoy	222
<i>Myrica Gale</i> , Note on the Distribution of Sexes in (Plate I and 1 Text-fig.)—A. J. Davey and C. M. Gibson	147
Myxophyceæ, <i>see</i> Algæ.	
Nutrient Solution in Water Culture Experiments, Obser- vations on the Influence of Aeration on the— Stiles, Walter, and Jörgensen, Ingvar	181
Osazone method of detecting sugars in plant tissues, On some criticisms of the—Sydney Mangham	236
Parasitism, On the Physiology of—William Brown ..	109
<i>Parnassia palustris</i> and <i>Valeriana dioica</i> , Radical Leaves of (2 Text-figs.)—H. Stuart Thompson	151
Peridineæ, <i>see</i> Algæ.	
<i>Pleodorina illinoiensis</i> , Note on—W. B. Grove	180
Physiology of Parasitism, On the—William Brown ...	109
Pollen Presentation Mechanism in the Compositæ, <i>see</i> <i>nuder</i> Small, James.	
Pteridospermeæ, <i>see under</i> Fossil Plants.	
Radical leaves of <i>Parnassia palustris</i> and <i>Valeriani dioica</i> (2 Text-figs.)—H. Stuart Thompson	151
Reconstruction of Elementary Botanical Teaching, The...	241
<i>Rhizophidium acuforme</i> Zopf (Fisch) (1 Text-fig.)—W. B. Grove	177
Sexes, Distribution of, in <i>Myrica Gale</i> , Note on (Plate I, and 1 Text-fig.)—A. J. Davey and C. M. Gibson	147
Spores of <i>Leptosphaeria acuta</i> , On the Forcible Discharge of (15 Text-figs.)—W. J. Hodgetts	139
Sugars in plant tissues, On some criticisms of the osazone method of detecting—Sydney Mangham	236
Syrphid Visitors to Certain Flowers, The—E. and H. Drabble	105
<i>Targionia hypophylla</i> , Note on—Shiv Ram Kashyap ...	228
Teaching, The Reconstruction of Elementary Botanical...	241
Transpiration, Recent Work on—Knight, R. C.	127
<i>Valeriana dioica</i> and <i>Parnassia palustris</i> , Radical leaves of (2 Text-figs.)—H. Stuart Thompson	151

Van't Hoff, <i>see</i> under Jörgensen, Ingvar, and Stiles, Walter.	
Water Culture Experiments, Observations on the Influence of Aeration on the Nutrient Solution in—Stiles, Walter, and Jörgensen, Ingvar	181
Weeds, The Effect of upon Cereal Crops—Winifred E. Brenchley	53
Willstätter, <i>see under</i> Jörgensen, Ingvar, and Stiles, Walter.	



INDEX OF AUTHORS.

	Page
Brenchley, Winifrid E. The Effect of Weeds upon Cereal Crops	53
Brown, William. On the Physiology of Parasitism ...	109
Davey, A. J., and Gibson C. M. Note on the distribution of sexes in <i>Myrica Gale</i> (Plate I and 1 Text-fig.)	147
Drabble, E. and H. The Syrphid Visitors to Certain Flowers	105
Dufrénoy, Jean. The Endotrophic Mycorrhiza of Ericaceæ (4 Text-figs.)	222
F(ritsch), F. E. Cambridge Botanical Text Books, Vol. I. Algæ (Myxophyceæ Peridineæ, Bacillariæ Chlorophyceæ). By G. S. West [Review]	48
Gibson, C. M., <i>see</i> Davey, A. J.	
Grove, W. B. <i>Rhizophidium acuforme</i> Zopf. (Fisch) (1 Text-fig.)	177
„ „ Note on <i>Pleodorina illinoiensis</i>	180
Hodgetts, W. J. On the forcible discharge of spores of <i>Leptosphaeria acuta</i> (15 Text-figs.)	139
Jørgensen, Ingvar, and Stiles, Walter. Carbon Assimilation: A review of recent work on the pigments of the green leaf and the processes connected with them:—	
Chapter VI. Energy relations in carbon assimilation—	
A. General remarks	24
B. Quantitative estimation of carbon assimilation by means of the products	25
C. Quantitative determination of the heat of combustion of the products of assimilation	30
D. Quantitative measurement of the radiant energy incident on the leaf and the utilisation of this energy ...	31
E. Assimilation in relation to radiant energy of different wave lengths ...	40
Chapter VII. Theories of Carbon Assimilation—	
A. General Remarks	77
B. Hypothesis of Baeyer	78

C. Suggestion of Van't Hoff	82
D. Suggestion of Siegfried	83
E. Theories of Willstätter	84
Chapter VIII. Concluding Remarks	91
Literature cited	94
Jørgensen, Ingvar, <i>see under</i> Stiles.	
Kashyap, Shiv Ram. Note on <i>Targionia hypophylla</i> ...	228
Knight, R. C. Recent Work on Transpiration	127
Mangham, Sydney. On some criticisms of the osazone method of detecting sugars in plant tissues	236
Sahni, Birbal. Observations on the Evolution of Branching in the Filicales (1 Text-fig.) ...	1
S(cott), D. H. Fossil Plants, Vol. III. Pteridospermeæ, Cycadophytes, Cordaitales, Cycad- ophyta. By A. C. Seward (Review)	230
S(eward), A. C. Ruth Holden (1890-1917)	154
Small, James. The Origin and Development of the Compositæ.	
Introduction	157
Chapter I. History of the Classification of the Compositæ (3 Text-figs.) ...	159
Chapter II. The Pollen Presentation Mechanism (4 Text-figs.)	198
Chapter III. Irritability of the Pollen Presentation Mechanism	253
Stiles, Walter, and Jørgensen, Ingvar. Observations on the influence of aeration on the nutrient solution in water culture experiments, with some remarks on the water culture method ...	181
Stiles, Walter, <i>see under</i> Jørgensen.	
T(homas), E. N. John William Hart	45
Thompson, H. Stuart. Radical leaves of <i>Parnassia</i> <i>palustris</i> and <i>Valeriana dioica</i> (2 Text-figs.)	151

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OBSERVATIONS ON THE EVOLUTION OF
BRANCHING IN THE FILICALES.*

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[WITH ONE FIGURE IN THE TEXT].

I. INTRODUCTION.

THE starting point of this paper was an attempt to see how far our conception of the branch as a potential individual¹ finds support from the recapitulation by the branch of the sequence of structural changes undergone by the main axis during its ontogeny. In this connexion a previous statement by the late Professor Gwynne-Vaughan² was particularly suggestive, namely, that "the ontogeny of the vascular system of the plant as a whole is very frequently repeated, although more or less imperfectly, in the development of its lateral branches"—a statement which is substantiated by the researches of Stenzel³ on several species of *Aspidium*, *Alsophila excelsa*, *Blechnum* and *Struthiopteris germanica*, and of Mettenius⁴ on various other dictyostelic Ferns; by those of Gwynne-Vaughan himself (*loc. cit.*) on a number of solenostelic and dictyostelic Ferns, and, more recently, by those of Professors Lang⁵ and Bower⁶ respectively on the Ophioglossaceæ,

*Dissertation presented for the Sudbury-Hardyman Prize, 1917, at Emmanuel College, Cambridge.

¹ Braun, Alex, Betrachtungen ü. d. Erscheinung d. Verjüngung in der Natur., 1851, p. 25.

² Gwynne-Vaughan, Annals of Botany, 1903, pp. 724-5.

³ Stenzel, Verhandlungen der K. Leop. Carol. Akad. der Naturforscher, 1861, p. 16.

⁴ Mettenius, Abhandlungen der kgl. sächs. Ges. 1864, Vol. IX. Ueber den Bau von *Angiopteris*, p. 499.

⁵ Lang, W. H., Annals of Botany, 1913, Vol. XXVII, i. p. 203 and 1915, Vol. XXIX, p. 1.

⁶ Bower, F. O., Annals of Botany, 1910, p. 423; 1912, p. 269; 1913, p. 443; 1914, p. 363; 1915, p. 495.

and on the Cyatheaceæ and Ferns of Cyatheoid affinity besides some other forms.

On taking a bird's-eye view of the subject it was observed that many of the forms fell into a rather striking series illustrating progressive stages in the specialization of the branch for the purpose of vegetative propagation. This progression appeared to be as clear and unmistakeable in respect of the vascular structure, as in respect of the external appearance of the branch: the two lines in fact ran parallel to each other and rendered more or less mutual support.

But the interpretation of the series in terms of a phylogenetic relationship between its different stages seemed to be a difficult question, for, as will be seen, the different genera did not *necessarily* stand towards each other in relations corresponding to those subsisting between the respective forms of branching exhibited by them.

II. TYPES OF FILICINEAN BRANCHES AND THEIR VASCULAR SYSTEM.

It is well-known that in many species of *Nephrolepis* thin cylindrical stolons arise from among the crowded leaf-bases and, spreading in all directions from the main stock, grow out to varying lengths, and serve as efficient organs of vegetative reproduction by bearing so-called lateral plants at intervals. Under suitable conditions the growth of the stolons is so rapid that in a single season the mother plant may become surrounded by a small colony of young plants which eventually become independent, but even before doing so may, in turn, produce stolons themselves. Sperlich¹ sees in the production of these stolons the beginning of an attempt towards the attainment of an epiphytic habit; and to what a degree this attempt has been successful is well seen in *Nephrolepis volubilis*. Attention was drawn in a previous communication² to the extraordinary habit of this interesting Malayan Fern. The mother-plant is rooted in the soil, but the stolons are of such a great length that they carry the lateral plants borne on them high up among the neighbouring trees. It is a case, in short, of a terrestrial plant producing an epiphytic progeny. The epiphytic tendency so strikingly shown by the genus *Nephrolepis* may possibly be connected with a more effective vegetative propagation which would be ensured by the favourable position of the lateral plants.

¹ Sperlich, A., Flora, 1908, p. 357.

² Sahni, B., NEW PHYTOLOGIST, 1915, p. 251.

The morphological nature of the stolons of *Nephrolepis* has in the past been a matter of controversy,¹ but even a superficial comparison with other Ferns which are known to possess more or less specialized lateral branches to the stem will convince one of their true nature; to Kunze (1849), to Hofmeister (1857) and to Mettenius (1861) they were so apparent as specialized shoots that the possibility of their being of a different nature did not even present itself to them.

It is, in fact, possible to trace the origin of the highly specialized condition in *Nephrolepis volubilis* downwards through a series of transitional stages found in other Ferns, to that in which the stem divides at the apex into two approximately equivalent branches. This mode of branching is very common among forms with a creeping rhizome (*Polypodium* spp., *Davallia* spp.), and a forking of the stem is occasionally seen even in some erect or semi-erect forms, e.g., in *Plagiogyria semicordata*.² But in many other Ferns, chiefly of erect or semi-erect habit, there seems to be a tendency on the part of the branches to become modified to serve as organs of vegetative reproduction. They undergo a marked elongation in their proximal portion, which may be underground and bears only reduced scale-like leaves, and which in its modified form may be called a stolon. The stolon takes a horizontal or oblique course for a longer or shorter distance away from the mother axis before it passes over distally into the leafy crown. It evidently serves to remove the leafy end of the branch away from the mother axis before the former can strike root and assert its individuality as a separate plant. Velenovský³ records in *Struthiopteris germanica* underground stolons as long as three metres; rather similar stolons have recently been described by Professor Bower in *Thyrsopteris elegans*, *Plagiogyria*, etc., while Stenzel long ago drew attention to the specialized branches of *Alsophila excelsa*. The genus *Nephrolepis* indicates a further advance in specialization, since the stolons are normally entirely leafless. Moreover, whereas further branching of the stolon is rare in the previously mentioned forms (so that usually

¹ For references see the paper just cited, p. 269.

Among Ferns, structures which in appearance as well as in function most closely correspond to the stolons of *Nephrolepis* are the so-called Ausläuferblätter (runner-leaves, leaf-runners) of *Asplenium obtusilobum* Hk., *A. Mannii* Hk., etc., described by Professor Goebel (Biologisches Centralblatt XXI, 1902, p. 385) and more recently by Dr. Kupper (Flora, 1906, p. 337). There can, however, be no question of a homology between the two: the Ausläuferblätter, as Professor Goebel showed, are morphologically leaves.

² Bower, *loc. cit.*, 1910, p. 426.

³ Velenovský, Vergleichende Morphologie, Prag, 1905, Vol. I, p. 232.

each stolon bears only one crown of leaves), in *Nephrolepis* the stolon has extensive powers of branching. The apex of the stolon does not itself produce a leafy crown, but during its continued growth produces a large number of secondary growing points, many of which sprout into "lateral plants"¹ while the rest of them remain dormant. In view of the enormous length of the stolons, which ensures a corresponding increase in the number of lateral plants, and in the epiphytic habit of the latter, *N. volubilis* would seem to represent a further stage in the specialization of the filicinean branch.

Turning now to the question from the point of view of the vascular anatomy, we find a series more or less parallel to the one we have based upon the external features. For our comparison we shall refer to Fig. 1, p. 5 which represents in a purely diagrammatic way the vascular relations of the branch to the stock in a number of Ferns whose mature structure ranges from protostely to an advanced type of dictyostely. In the figure the branches are supposed to have come off in all cases at right angles to the main axis, so that they can be drawn as seen in longitudinal section while the main axis is seen in transverse section. In the branch the protostelic portion is shown as a single black band arising from the main axis; the solenostelic portion is shown as two bands which are parallel except towards their proximal ends, where the solenostele is seen to contract. In the solenostelic portions (whether of the main axis or of the branch), the leaf-gaps are not indicated, for the sake of better distinction from the reticulate portions, which are represented by interrupted bands. The leaf-traces have been omitted altogether, and no attempt has been made to draw the diagrams to scale.

Some of the diagrams would require further explanation in view of the facts that the branch very frequently arises in close association with a leaf, in an "axillary" or "infra-axillary" position, and that its vascular supply appears actually to originate from the trace of the related leaf, as in some Hymenophyllaceæ, species of *Zygopteris*, in *Cibotium*, *Plagiogyria*, *Lophosoria*, *Metaxya*, *Cheiropleuria* and *Botrychium Lunaria*. Without entering here into the question of the morphology of the organ in *Zygopteris* termed by Dr. Scott the "undivided trace," it may be stated that the view here adopted regards the branch as arising from the main axis, but with

¹ Thus with respect to the mother plant the lateral plants are usually branches of the second order. If, however, the apex of the main stolon is arrested, it is replaced by one of the neighbouring secondary growing points.

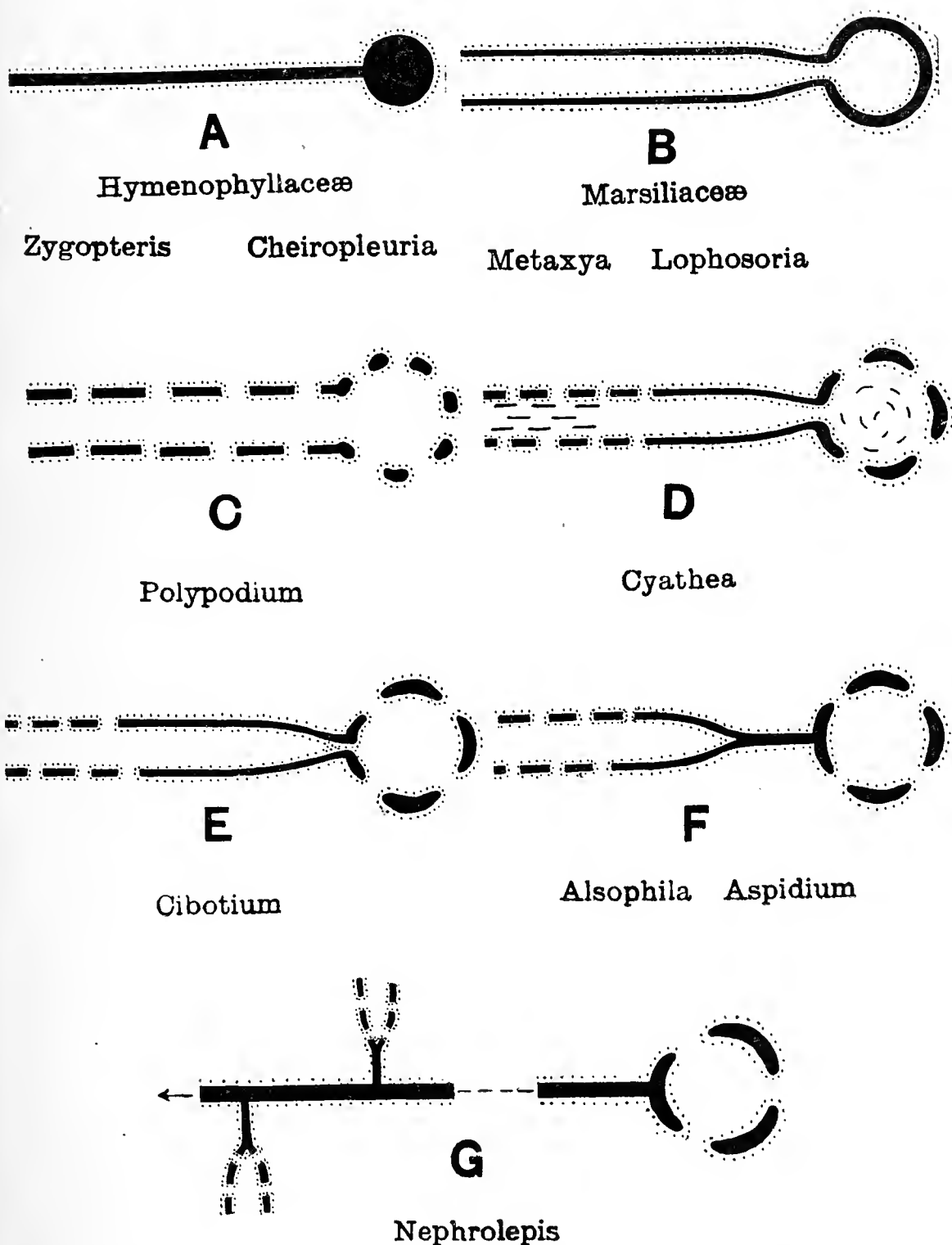


Fig. 1. Diagrams to illustrate the vascular relations between branch and main axis. *Xylem* black, *phloem* dotted. The thinner lines in D represent the medullary strands. Further explanation in the text, p. 4-9.

the proximal portion of its strand "adherent" to the trace of the associated leaf, which belongs to the main axis.¹ The strand under discussion would thus have a composite nature, and we may extend

¹ A view which we owe originally to Dr. Scott, (*Annals of Botany*, 1912, Vol. XXVI, i. p. 59) but which was rejected by him in preference to the leaf-trace theory. The question is further dealt with on p. 19 of the present paper

this view to all cases discussed in this paper, in which a branch arises in close vascular connexion with a leaf. In accordance with this view the plane of section in Fig. 1, A, B, D, E must be supposed to have passed, at the base of the branch, longitudinally through the ramular portion of this composite trace, the foliar portion of the latter being proximal or distal to the plane of section according as the branch is adaxial (Hymenophyllaceæ, *Zygopteris*, *Plagiogyria*, *Botrychium*) or abaxial to the leaf (*Cibotium*, *Lophosoria*, *Metaxya*, *Cheiropleuria*).

In the protostelic forms the branch always receives, as would be expected, a single solid strand which usually remains protostelic throughout its length (Hymenophyllaceæ,¹ *Zygopteris*, *Cheiropleuria*, Fig. 1, A). The branching may be either distinctly lateral, as in *Cheiropleuria* and the Hymenophyllaceæ, or a forking of the axis, as in *Zygopteris* (*Ankyropteris*) *corrugata*,² *Diplolabis Ræmeri*, *Metaclepsydroopsis duplex*,³ *Botrychioxylon paradoxum*.⁴

In the solenostelic forms we find that the vascular system of the branch frequently appears to arise as a diverticulum of an associated leaf-trace, (*Lophosoria*, *Metaxya*) but, as explained above, may be regarded as being fused along one side of the base of the latter. In a number of solenostelic forms investigated by Gwynne-Vaughan, the branch at its base has a gutter-shaped strand, which, however, rapidly closes up into a complete cylinder (several species of *Hypolepis*, *Polypodium punctatum*, *Dicksonia adiantoides*). In the Marsiliaceæ with a tubular stele in the main axis the branch receives also a tubular strand, the pith of the branch being continuous through a gap with that of the main axis.⁵ According to Professor Seward,⁶ in *Matonia pectinata* the rhizome divides by forking, and sends into each branch a solenostele, as well as a portion of the accessory medullary system.

The branching of *Helminthostachys* has recently been described in detail by Professor Lang (*loc. cit.* 1915), and although the plant does not possess a typical solenostele it may be considered here. The branches arise from dormant axillary buds which are stimulated

¹ See, however, p. 14 below, in reference to *Hymenophyllum lineare*.

² Scott, *Studies in Fossil Botany*, 1908, p. 318.

³ Gordon, *Trans. Roy. Soc. Edinb.*, Vol. XLVII, Part IV, p. 720 and Vol. XLVIII, Part I, p. 173.

⁴ Scott, *Trans. Linn. Soc.*, 2nd ser. Botany, Vol. VII, Part XVII, 1912, p. 383.

⁵ De Bary, *Comparative Anatomy*, English Translation, 1884. p. 313.

⁶ Seward, *Phil. Trans.*, 1899, Vol. CXCI, p. 187,

to activity when the growth of the apex of the main rhizome is arrested. The attachment of the branch stele is directly to the main stele, a short but variable distance in front of the related leaf-trace. There is some variation also in the behaviour of the vascular system of the branch, but in general it may be said that the branch supply originates as a single strand which rapidly becomes curved and then closes up into a xylem tube enclosing a "mixed" central xylem. The parenchyma sooner or later replaces the central tracheides altogether, forming a definite pith.

It is when we come to the Ferns with reticulate steles, however that we find the greatest diversity in the mode of origin of the branch vascular supply. In a large number of cases the vascular system of the branch takes its origin as a single protostelic strand although the main axis in that region is dictyostelic (several spp. of *Aspidium*, *Alsophila excelsa*, Fig. 1, F, *Struthiopteris germanica* and *Nephrolepis*). The protostele arises from one of the cauline strands, and persists for a varying distance outwards before it expands in a funnel-like manner into a solenostele, this giving place sooner or later to a dictyostele. In *Nephrolepis* the protostelic stage is enormously long, being met with throughout the length of the stolon; in this genus, moreover, the main protostele does not itself expand into a solenostele, but gives rise to secondary protosteles which enter the bases of the lateral plants. There each expands first into an extremely short funnel-like portion immediately succeeded by a dictyostele (Fig. 1, G). The branch supply in *Struthiopteris germanica* originates, according to Stenzel, as an abaxially grooved strand which, traced distally, is seen to close up into a complete tube. This tube further on becomes perforated by the leaf-gaps, so that from this point onwards the branch has a stele exactly like that of the main axis. Secondary branches are recorded by Stenzel in this species.

In *Aspidium* and *Alsophila excelsa* the protostelic stage is very short, being in the latter species succeeded by the solenostele near the point of exit of the branch-stele from the cortex of the main stem. Stenzel describes in the same plant one case in which the protostelic region was altogether unrepresented, the funnel-like expansion being sessile on the large cauline strand.

A more interesting condition is recorded by Professor Bower (*loc. cit.*, 1913, p. 454 and pl. 33, fig. 13) in the stolon of *Cibotium Barometz*, the stele of which at its base contracted to the *Lindsaya*-stage, so that its phloem was continuous, through a gap in the

xylem, with the internal phloem of the main stele.

The branch stele in *Adiantum trapeziforme*, which has a dictyostelic adult stem, is at its base a completely closed tube, having its pith continuous with that of the main axis (Gwynne-Vaughan, *loc. cit.*, p. 724). In some of the Cyatheæ we meet with a similar condition, which may be somewhat complicated by the presence of a medullary system of strands, both in the main axis and in the branch. The branch may either develop its medullary system independently of that in the main axis, or the two may be continuous through the gap at the base of the branch. The latter condition is described by Stenzel in a Fern erroneously called by him *Diplazium giganteum*.¹ In *Hemitelia setosa* (Klf.) Mett. Professor Bower records a branching stolon—the main stolon corresponded with the mother axis in its advanced type of Cyatheoid structure, while the secondary stolon was solenostelic, though it still possessed a weak medullary system.

In some material of a Malayan species of *Drymoglossum*, for which I am indebted to Mr. F. T. Brooks, the branch possessed at its base a C-shaped strand with its gap adaxial. This strand almost immediately broke up into two, which divided further so as to form a reticulate stele exactly like that in the main axis.

Lastly, we may mention the comprehensive genus *Polypodium*, among a large number of other Ferns with highly dissected steles and creeping rhizomes. In a great many of these the branch at its very base is dictyostelic, and in external appearance does not differ from the main axis, except perhaps in size. Thus Klein² describes in *Polypodium Heracleum* side branches which are attached to the main axis by a narrow base; the dictyostele in such branches is correspondingly contracted at its insertion on that of the main axis, and the first one or more leaves of the branch may fail to attain their normal stature. In *P. quercifolium* the same author describes both branches growing out from a contracted base and possessing a miniature dictyostele which increases in diameter with the branch itself; and others which are from the very start identical in structure with the main axis. These latter cases are not far removed from dichotomy in the wider sense. Here we may refer also to the Osmundaceæ, in which dichotomy of the rhizome has been described

¹ The genus *Diplazium* is generally placed in the proximity of *Asplenium* and *Scolopendrium*, but the description of the stem and petiole of Stenzel's Fern points unmistakably towards the typical Cyatheaceous structure.

² Klein, L., Nova Acta Leop.-Carol. Deutschen Akad. d. Naturf., Bd. XLII No. 7, p. 335 ff., 1881.

by Faull.¹ Immediately beyond the bifurcation either each branch may possess a complete ring of separate xylem strands (*Osmunda Claytoniana*), or the two rings may be interrupted by ramular gaps on the sides facing each other (*O. cinnamomea*, *O. regalis*, *Todea barbara*).

We have now reviewed the vascular relations between branch and main axis in a large number of Ferns selected from nearly all the great families in which the branching of the stem has been described and ranging in stelar structure from the simplest to the most complex type. The results may be briefly summarized as follows:—

I. In Ferns with a *protostelic* main axis the branch is also as a rule protostelic throughout its length (Hymenophyllaceæ, *Zygopteris*, *Cheiropleuria*) Fig. 1, A.

II. Ferns possessing a *tubular stele* in the main axis have in their branches either a tubular stele throughout their length (Marsiliaceæ, *Lophosoria*, *Metaxya*, *Hypolepis*, *Pteris incisa*, var. *integrifolia*, etc., Fig. 1, B) or at the base a solid strand which soon becomes converted into a tube (*Helminthostachys*).

III. In Ferns which have a *reticulate stele* in the main axis the vascular system of the branch may either

(a) be from the very base a reticulate stele, which may or may not be attenuated towards its insertion to the main stele (Osmundaceæ, *Polypodium* spp., Fig. 1, C) or

(b) it may start at the solenostelic stage, also often contracted at the base (*Adiantum trapeziforme*, *Cyathea*, etc., Fig. 1, D) or

(c) it may possess at its base the *Lindsaya*-type of structure (*Cibotium Barometz*, Fig. 1, E) or, lastly

(d) it may begin with a thin protostelic strand which is of variable length (*Alsophila excelsa*, *Aspidium cristatum*, *A. spinulosum*, *Struthiopteris germanica*, *Nephrolepis*).

When the main axis contains a medullary system of strands the branch may either develop its medullary system independently of it, or the two may be continuous through the gap at the base of the branch (several *Cyatheæ*, *Polybotrya Meyeriana*, *Matonia*).

In each case except (a), the branch, during its growth, passes successively through morphologically higher and higher stages, till it acquires the type of stele characteristic of the adult main axis in that species.

¹ Faull, Bot. Gaz., Vol. XXXII, 1901, p. 398.

III. THEORETICAL CONSIDERATIONS.

a. The Primitive Branch.

Although the branching of Ferns has long been a subject of detailed investigation, and nearly all the different forms of the vascular relations between stem and branch described above have been known since the publication of De Bary's "Comparative Anatomy," it has not hitherto been suggested that these relations can be arranged in such a regular series as that shown in Fig. 1, and the interpretation of the series in terms of the phylogeny of the filicinean branch has consequently not been attempted. Gwynne-Vaughan was the first to suggest that the ontogeny of the vascular system of the main axis is often more or less imperfectly repeated in the development of the branch; and subsequent work, chiefly that of Professor Bower, has added considerably to the data on which that suggestion was originally founded. It still remains to be seen which of the two conditions is the more primitive for the branch—is it that in which this repetition is more perfect, in which the branch, starting life with a thin protostelic strand, gradually "works its way up" to the morphological level of the main axis; or that in which it is from the very beginning at or near that level? These two conditions are represented by the two ends of the series C—F; the ends are connected by means of stages morphologically intermediate between them; and the problem before us is to decide whether the series is a descending or an ascending series.

It is evident that this problem is inseparably connected with the wider and much debated question of the primitiveness or otherwise of dichotomous as opposed to monopodial branching, and that the answer to the first will give the clue to the second.

Before proceeding to our discussion, the conclusion arrived at may be stated at once, namely, that the forms of branching depicted in Fig. 1, C—F, constitute an ascending series. In our discussion we shall give particular attention to the branching of the higher Ferns (those with reticulate steles), as affording a more complete illustration of the parallelism in structure between stem and branch, and a greater variety in the behaviour of the branch vascular system.

Naturally, the most direct way to attack the problem would be to enquire into the circumstances which lead to the production of the respective forms of branching. Let us conceive of the growing stem of a dictyostelic Fern which is about to branch, and ask

ourselves what are the conditions necessary to ensure the production of a branch fundamentally similar in structure to the original axis. Evidently a fundamental similarity of growth-conditions for the two resulting growing points is the essential provision; granted this the two growing points will inevitably produce two essentially similar (dictyostelic) branches, and according to the degree of similarity in the growth conditions the form of branching will more or less closely resemble a dichotomy. However, when the conditions are fairly unequal the resulting branches may no longer be to one another as the two arms of a fork. One of the arms may "tend to push the other aside and approximate to a continuation of the original axis."¹ In time the one comes to be known as the "main axis" "giving off" the other as a branch, though both may in their vascular structure be dictyostelic. The dichotomy may pass insensibly into the monopodial type of branching.

We have only to go a few steps further to arrive at the other extreme of our series, Fig. 1, P. It is a common occurrence for one of the products of division of the growing apex to become dormant *almost immediately* after its formation, while the other continues its growth as the main apex of the plant. This state of affairs is undoubtedly more specialized than, and easily derived from, that in which the two apices continue to grow side by side both utilizing to about the same degree the conducting system in their rear. One of the apices has been sacrificed to the better nourishment of the other, by passing over into a state of temporary inertia, and provision is thereby also made for a resumption of activity in case the main apex meets with an accident or otherwise becomes checked in its growth.²

¹ Tansley, "Lectures on the Evolution of the Filicinean Vascular System," NEW PHYTOLOGIST, 1907, p. 29.

² Such dormant buds, which trace their origin to the growing point, have to be distinguished from so-called adventitious buds, which appear in no constant position and as the result of a secondary meristematic activity in tissues already more or less mature. The former may conveniently be termed "primary" buds in contrast to the adventitious or "secondary" buds. The ultimate distinction, however, is only developmental, and even that criterion would be difficult to apply in the case of adventitious buds arising near enough to the growing point, but the latter condition appears not to have been recorded. According to Sadebeck's observations (Schenk's Handbuch I, 1881, p. 266) the adventitious buds in Ferns appear to be confined to the leaves. Sadebeck mentions as an exception to this statement the buds in the Ophioglossaceæ. Professor Lang, however, concludes from his detailed researches that the buds on the stem, at least, of *Botrychium Lunaria* and *Helminthostachys* are "part of the primary construction of the plant," and not adventitious as Farmer and Freeman (Annals of Botany, 1899, Vol. XIII, p. 423) had previously stated.

From several other sources similar conclusions have come to light regarding the buds found in relation to the branching of Ferns. Thus, Hofmeister's view (Beiträge etc. II, Abhandlungen der Kgl. Sächs. Ges. der

Such primary dormant buds are commonly seen on Fern stems, and as we have seen, very frequently in the proximity of leaf-bases. In accordance with the insignificant demands that they make on the food resources of the plant so long as they are in a dormant condition, a single thread-like strand is seen to connect them to the main vascular system, although the latter may be solenostelic or even dictyostelic. But when a plant has reached a certain development the surplus energy is diverted into these side channels. In response to the increased flow of food and water the thin strand at the base of the young branch is succeeded distally by a stouter and more efficient conducting system. If the adult stem has a protostele in the main axis, the branch usually retains a protostele; if the main axis is soleno- or dictyostelic the vascular system of the branch expands accordingly. A branch of a dictyostelic Fern, for example, produced in these conditions, would correspond to what we find in *Alsophila excelsa*, *Aspidium cristatum*, *A. spinulosum* (Fig. 1, F).

At this point the question may reasonably be asked, why is it that in the case of a soleno- or dictyostelic Fern the dormant bud does not always establish a diminutive soleno- or dictyostelic connexion with the main axis, rather than a protostelic one? We must remember that we have hitherto dealt only with buds which became dormant almost immediately after their origin. This is not always the case, however. The dormant stage may be delayed for a longer or shorter period after the division of the main growing point,¹ so that for a time the products of division have a more or less equal share of the food resources, and naturally develop a similar vascular system. This is, in fact, what happens in *Polypodium vulgare* according to Klein's observations,² and Stenzel's figures of the same species (*loc. cit.*, 1861, pl. V) show the reticulate stele of the main axis giving off similar steles to the branches. Klein's observations on *P. Heracleum* and *P. quercifolium*³ are further examples of the same phenomenon.

Wiss. V, 1857, Math.-Phys., Klasse III, p. 651) that the stolons of *Nephrolepis* arise from adventitious buds was shown to be incorrect by Sperlich in 1906 (Flora, p. 469). Further, Sadebeck's statement (in Engler u. Prantl, Nat. Pflanzenfam. I, 4, 1st part, p. 44) that the branching of Ferns with dorsiventral rhizomes is due to lateral buds traceable to the main apex, is in support of the general view expressed in 1861 by Stenzel (*loc. cit.*, p. 34) that the branching of the Fern stem has no relation to adventitious buds. Pringsheim appears to have been the first to express this opinion (Bot. Ztg., 1853, p. 609), which was in 1855 adopted by Irmisch (see Hofmeister in Pringsheim's Jahrb., Vol. III, 1863, p. 279). If this is true, as seems probable, there is no doubt also that the buds in *Cheiropleuria*, *Lophosoria* and other Ferns investigated by Professor Bower, are of primary origin.

¹ Schoute, Ann. Bot., 1906, p. 88.

² Klein, L., Bot. Ztg., 1884, p. 585.

³ Klein, Nova Acta, 1881, Bd. XLII, pp. 353, 366.

Thus the development of a thread-like or reticulate branch-stele, as the case may be, from a reticulate main stele, would seem to depend upon whether the rudiment of the branch became dormant soon after its first origin or whether it was allowed for some time to grow side by side with the apex of the main axis before it became quiescent. The extremely minute size of the branch-initial in the former case might explain the thread-like character of the stele—a kind of stele which would result if the contraction of a reticulate stele be carried far enough. In this connexion it is rather illuminating to notice that even when a reticulate main stele gives off a similar branch stele, the latter is often rather suddenly constricted at the base, though still reticulate. This constriction is obviously due to the fact that the branch-bud was at an early stage pushed aside, though not at once reduced to dormancy, by the main growing point so that the development of the two was unequal at first.

We have tried to analyse the biological conditions governing the two extreme modes of branching shown in Fig. 1, C, F. More difficult seems an explanation of the intermediate stages, D, E, where from a dictyostelic main axis arise branches which at their insertion are respectively at the solenostelic and the *Lindsaya*-stages. We lack the support of observed facts, and no definite statement can be made till further work has been carried out. At the same time, there seems to be nothing against our explaining these cases on the same principle as above, namely, that the formation of the type of vascular cylinder is determined by the size of the bud at the commencement of its activity, this size being more or less fixed for the given species and controlled by the biological conditions.

That the conditions of nutrition exercise a far-reaching influence on the development of the vascular system is only an expression of the general principle that these conditions are all-important for the development of the whole plant—a principle which is the foundation stone of the science of experimental morphology. We shall consider a few examples which appear to be particularly instructive in connexion with the problem before us.

In his study of *Matonia pectinata* Mr. Tansley¹ described some plants which were found in a deeply shaded rock-crevice. To quote his own words, “they were, no doubt, plants of some age which had been unable to attain anything like their full growth owing to

¹ Tansley and Lulham, *Annals of Botany*, Vol. XIX, 1905, p. 490.

want of soil and light. Their vascular system is of interest, since it leads up from that of the . . . young plants to that of the typical adults." In some plants of *Helminthostachys zeylanica* Professor Lang¹ found that the rhizome, which had already attained the adult type of structure (with tubular xylem), subsequently reverted to the juvenile (protostelic) condition by passing through a series of changes involving a diminution in size of the whole rhizome. Professor Lang legitimately explains the phenomenon as due to growth under less favourable conditions of nutrition. It is of interest to find the same view expressed by Professor Bower in his paper on the origin of medullation in the Pteridophyta.² He remarks that in the Ophioglossaceæ the young plant may at first either have a solid xylem cylinder, or there may be a small pith from the very beginning; and he relates the latter condition to a more efficient nutrition of the young plant.

In their development such young plants are directly comparable with the different types of branches described above, whose steles at the base are medullate or non-medullate according as their rudiments entered at once on a vigorous life, or, on the other hand, became dormant while still relatively minute, and subsequently had to work up from a small beginning. In striking consonance with this idea is Professor Lang's conclusion (*loc. cit.*, 1915, p. 34) that "the simpler type of stele characteristic of normal young stages in the ontogeny of *Helminthostachys* is to be associated with small size and less efficient nutrition." This conclusion may well be extended to all vascular plants developing from a small beginning. As the flow of food increases the axis grows in thickness, and its stele undergoes a corresponding dilatation; we have here a phenomenon similar in essentials to that seen in the tubers of *Nephrolepis*³ though less pronounced in degree.

I regret that when I wrote the paper just cited I was not aware of the papers referred to in the footnote.⁴ Of these the last-mentioned requires special attention. The author describes, in *Hymenophyllum lineare*, sessile or stalked tuberous bodies produced as side-branches from the rhizome. From the description and the

¹ Lang, *Annals of Botany*, 1915, p. 33.

² Bower, *Annals of Botany*, 1911, p. 550.

³ Sahni, B., *NEW PHYTOLOGIST*, 1916, p. 72.

⁴ Senn., *Verh. natf. Ges. Basel*. Bd. XXI, p. 115, *Die Knollen von Polypodium Brunei* Werckle; Christ und Giesenhagen, *Flora*, 1899, p. 79.

Giesenhagen, *Berichte d. deutschen bot. Ges.*, 1909, p. 331 and Pl. XV, fig. 8.

figure given it appears that a thin protostelic strand branches off from the main stele and, on entering the base of the tuber, expands and acquires a pith. It is not said whether it becomes converted into a reticulate stele. Seeing that the adult rhizome of *Hymenophyllum* never possesses a medullate stele, the appearance of such a structure in the tuber carries a peculiar theoretical interest, not only as supporting the hypothesis offered in my paper on the tubers of *Nephrolepis*, but also as showing that under the influence of physiological factors an organ may, so to say, break loose from the morphological check which may be said to have been imposed upon it by the phylogenetic position of the plant.

To take an instance from the animal world, the governing influence of the conditions of nutrition on growth is well illustrated by the rather startling results which Gudernatsch¹ has recently obtained by feeding tadpoles respectively on the thymus and thyroid glands of various mammals. While young tadpoles fed on thyroid cease further growth in size, prematurely pass on to the changes of metamorphosis, and become dwarf frogs, those fed on thymus grow into giant tadpoles, and if they are kept long enough under this influence, the metamorphosis is altogether suppressed.

If the line of argument traced above is sound, we have in it a strong support for the view that in the Ferns at least the primitive type of branching was the dichotomous, and that the monopodial type was derived from it by the gradual suppression of the growing point of one of the arms of the fork, which in the extreme cases became dormant immediately after its origin. The impetus for the departure from dichotomy probably originated in the need for a more effective nourishment of one of the branches. This need was supplied at the expense of the other branch, whose further development was postponed till a surplus of nutrition was available. The most important line along which the latter branch has evolved is, as we have seen, specialization for vegetative propagation. As of secondary importance may be mentioned the modification of the branch to serve as a climbing organ, so well seen in *Nephrolepis*, as well as its transformation into a tuber for the storage of food and water (*N. tuberosa*, *Polypodium Brunei*).

Further, if the sequence of events has been as we have conjectured, we arrive at the interesting theoretical conclusion that the evolution of the filicinean branch has mainly consisted in the

¹ Gudernatsch, Zentralblatt für Physiologie, 1912, Vol. XXVI, p. 323.

intercalation, at its base, of a series of stages each morphologically less complex than the preceding; that, in short, the process has been one of retrogressive evolution in a basipetal direction. The appearance of relatively simple types of vascular structure at the base of a highly evolved branch, such as that in *Alsophila*, *Nephrolepis*, etc., must be regarded as a cœnogenetic feature, and not as strictly primitive.

In our attempt to trace the evolution of branching in Ferns we must constantly keep in view one fact, which was pointed out in the introduction to this paper. This was, that many Ferns of a relatively high order of evolution, such as *Polypodium* and *Pteridium*, commonly branch dichotomously, while on the other hand, relatively primitive types such as the Ophioglossaceæ may show a monopodial type of branching. Thus we do not find that there is a necessary parallelism between the evolution of the modes of branching on the one hand, and the evolution of the plants themselves on the other. We are driven to the conclusion that these two lines must have been independent of each other.

b. The Branch as a Potential Individual.

If, as we have suggested above, the evolution of the branch has in the main been guided by the need for vegetative propagation—and, of course, the phenomenon of branching in itself primarily implies an amplification of the plant-body—it is of interest to find that the further the branch recedes from its ancestral form, the more nearly does it approximate to our conception of it as a potential individual. For the sake of an ampler illustration we shall again confine ourselves to the branching of the dictyostelic series C—F, Fig. 1. We see here that the stele of the branch of *Alsophila excelsa*, in virtue of its origin from a relatively minute dormant bud, has necessarily to pass, during its growth, from a fine thread-like beginning, through gradually expanding solenostelic stages, before it finally attains the adult type of structure. Stenzel's description of the branch stele of *Alsophila* corresponds in the broad features with Gwynne-Vaughan's description of the ontogeny of the main stele. In sharp contrast stands the branch of *Polypodium* which, having at its disposal from the very beginning the ample food resources of the plant, has been able at the outset to develop a dictyostelic vascular system. A striking illustration of the same phenomenon was recorded in this journal,¹ where it was suggested that probably because of the more favourable physiological conditions in which it

¹ Sahni, B., NEW PHYTOLOGIST, 1915, pp. 263, 271.

begins its development the lateral plant of *Nephrolepis cordifolia* in its ontogeny omits some of the earlier stages in stelar evolution, which are passed through normally by the sporeling. It would be agreed that, inasmuch as it passes through a series of stages parallel to that passed through by the main axis during its ontogeny, the lateral branch of *Alsophila excelsa* more nearly corresponds to our idea of an individual, than does the branch of *Polypodium*.

We have taken as one criterion of individuality the degree to which the ontogeny of the branch resembles that of the corresponding main axis. Hence in a case like *Alsophila excelsa*, where this resemblance is rather close, the structure of the branch should be susceptible of the same phylogenetic interpretation in the light of the recapitulation theory, as the structure of the developing main axis. And this view should hold in spite of the probability that the simpler structure of the branch at the base is of relatively recent origin, because the *ontogeny* of the branch still resembles the ontogeny of the main axis.

The specialization of the branch has, however, gone further than the stage of *Alsophila excelsa*. The branch in *Nephrolepis* (Fig. 1, G) has been subjected to physiological conditions very different to those affecting the main axis. The result is that a portion of the branch, the stolon, has assumed an appearance and structure so widely different from that of the leaf-bearing stock, that anything short of an organic connexion between the two would not have convinced one that they both belong to the same plant. The apex of the primary stolon is endowed with the power of long-continued growth, and of producing a large number of lateral dormant buds, each the beginning of a potential individual.

c. The Relation Between Branch and Leaf.

We may pass now to another aspect of the evolution of the filicinean branch, namely, the relation of the branch to the leaf, which is so conspicuous a feature in relatively wide circles of affinity. We know that this relation existed in some of the most ancient known branching Ferns, the Zygopterideæ, and it is seen to-day in the Ophioglossaceæ Hymenophyllaceæ, Cyatheæ, and sporadically among Ferns of a still higher order.

It is to be noticed that the relative positions of branch and leaf vary in different groups of Ferns. The branch (or the rudiment of it which may fail to develop) is sometimes adaxial (Ophioglossaceæ, Hymenophyllaceæ) sometimes abaxial (Cyatheæ) to the

leaf, while in other cases it may apparently arise from the petiole of the related leaf, some distance from its base, either on the adaxial or the abaxial face.

Moreover, although in a general way the relative positions are fairly constant for each group taken by itself, this constancy tends to be discounted when we consider the vascular relations of the branch and leaf. In those Hymenophyllaceæ in which "axillary branches" are generally found it sometimes happens that the branch-trace is inserted on the main stele independently of the leaf-trace. Boodle¹ records one such case in *Trichomanes radicans*, and I have myself seen other cases in herbarium specimens. Mettenius² also draws attention to the varying position of the branch bud in the Hymenophyllaceæ. We have already referred to Professor Lang's statement (see p. 1) that the branch-trace in *Helminthostachys zeylanica* is inserted directly on the main axis, some distance in front of the trace of the subtending leaf. In the closely allied species *Botrychium Lunaria*, however, the same author found that whereas usually the chief vascular connexion of the branch was with the adaxial face of the leaf-trace, in some cases the attachment was deeper in the axil and more closely related to the stele of the main rhizome. Within the Zygopterideæ also we meet both with "axillary branches" having a vascular connexion with the trace of the subtending leaf, and with branches having their steles directly inserted on that of the main axis.

Nor do we find that the numerical relation of one branch to one leaf is obligatory, more than one branch to a leaf having been recorded not only in several species of *Hypolepis*, but also in *Cyathea mexicana*, *Dicksonia* (*Cibotium*) *Barometz*, *Polypodium grandiens*, *Cystopteris montana* and other Ferns.

Finally, considering the Ferns hitherto unmentioned we cannot detect in the great majority of them any relation whatever between the positions of branch and leaf.

Thus if we view the Ferns as a whole it may be said that the branch does not hold any regular position with respect to the leaf; and the question arises whether the primitive condition was that in which leaf and branch were separate, or that in which they were produced in relation to each other. From his detailed study of *Botrychium Lunaria* and *Helminthostachys zeylanica* Professor Lang has concluded that the regular presence of axillary buds in these species is in favour of this relation being "part of the

¹ Boodle, *Annals of Botany*, 1900, p. 473.

² Mettenius, *Abhandlungen der Kgl. Sächs. Ges.*, 1861. Ueber Seiten-Knospen bei Farnen, p. 615.

primary construction of these plants" (*loc. cit.*, 1915, p. 47). With this condition as a starting point he has postulated a gradual retreat of the branch from its subtending leaf, as well as a stronger development of the branch, to arrive at the dichotomous type of branching now known to be so common in the *Zygopterideæ*.

While it is agreed that in the *ontogeny* the axillary buds in the *Ophioglossaceæ* (and elsewhere) are laid down at the growing point of the axis, and are not adventitious, it seems difficult to admit that the production, by the growing point, of a branch rudiment regularly in connexion with each leaf rudiment, was the *primitive* state of affairs. The latter condition naturally suggests itself as being a specialized case of that in which there was no such regularity; and I venture to suggest that the origin of this relation, which is admittedly very ancient, may perhaps be traced to some possible biological advantage accruing from it. One of these was probably the protection of the young bud during its period of dormancy. However, while the protection afforded to the branch initial by its axillary position is obvious in such cases as *Zygopteris*, the *Hymenophyllaceæ*, and the *Ophioglossaceæ*, some other explanation must be sought for cases where the bud is abaxial to the leaf.

In his well-known recent paper on *Zygopteris Grayi* Dr. Scott discussed the morphology of the organ conveniently termed by him the "undivided trace."¹ Of the two views discussed, the one that regards the associated leaf as the first leaf of the branch, may be dismissed at once, not only in view of the variation above mentioned, but also because the leaf is always orientated with regard to the main axis, whether the branch is adaxial or abaxial to the leaf.

From the form and structure of the undivided trace in the Shore specimen of *Zygopteris Grayi* Dr. Scott has been led to decide in favour of the second view, which regards the strand in question as being from its base upwards of a foliar, and not axial, nature. However, as he himself suggests, "we may . . . regard the axillary stele as 'adherent' to the adaxial side of the leaf-trace" (*loc. cit.*, p. 59), and this is the position here adopted. While it is true, as Dr. Scott remarks, that this way of looking upon the subject does not throw any new light on the facts, and the difference is ultimately one of words, from a comparative

¹ Scott, *Annals of Botany*, 1912, Vol. XXVI. (i), pp. 57—60. Since the branching of *Zygopteris Grayi* and the *Hymenophyllaceæ* is analogous to that in the other Ferns in which the branch is closely related to the leaf, the term "undivided trace" may be extended to the corresponding organ in these cases, whether the branch is adaxial or abaxial to the leaf.

study of the corresponding structures in the living Ferns it seems more natural and therefore preferable to regard the strand in all such cases as being of a dual nature. The relative importance of the leaf and branch portions of the strand are subject to variation. In the figured Williamson specimens of *Zyopteris Grayi* the axial characters predominate, while in Dr. Scott's specimen the leaf-trace characters are more prominent, though still there is, in the latter specimen, a fairly well-developed adaxial bulge with its median strand of "mixed" internal xylem, destined to supply the branch. A similar variation in the relative importance of leaf and branch has been noticed by Professor Bower in *Plagiogyria* (*loc. cit.*, 1910, p. 434).

Professor Bower's account of the branching of *Cheiropleuria bicuspis*¹ is particularly illuminating in connection with this question. When the leaf has no branch in connection with it, its trace arises as a small tangentially flattened strand which divides into two before entering the petiole. When there is a branch connected with the leaf, a very much larger circular strand is given off from the main stele. Evidently this strand cannot correctly be described as the leaf trace, for at the proper level two small strands come off from its inner-face, and these constitute the divided part of the leaf-trace, while the bulk of the original strand passes into the "infra-axillary" branch. Since the leaf belongs properly to the main axis, the basal undivided portion of its trace must be regarded as being adherent to the inner face of the ramular portion of the "undivided trace."

The branching of *Pteridium aquilinum* still remains a puzzling case, in spite of the fact that this is such a common Fern, and has so frequently been investigated since the time of Hofmeister. No completely satisfactory interpretation can be given of the morphology of the vascular system of branch and leaf in this species, by any of the views hitherto offered. The more recent observations are those of Velenovsky,² Bayer,³ and Mr. Tansley,⁴ but only the last-mentioned author has taken account of all the different forms of branching observed in this species. It appears, however, that the only way to arrive at a definite solution, namely, a study of the development of the plant, has still to be carried out.

Mr. Tansley also described in *Pteris incisa* var. *integrifolia*, a

¹ Bower, *Annals of Botany*, 1915, Fig. 7, p. 505.

² Velenovsky. *Sitzungsber. Böhm. Ges. Prag.*, 1890 (Review in German, *Bot. Centralblatt*, Vol. 46, 1891, p. 32),

³ As referred to in Velenovsky, *Vergleichende Morphologie*, 1905, Vol. 1,

⁴ Tansley and Lulham. *New Phytologist*, 1904, p. 1.

dichotomy of the rhizome with a petiole coming off exactly at the angle and forming a direct continuation of the axis behind the angle. As he pointed out, this case finds the closest analogy in *Hypolepis tenuifolia*, the chief difference being that the branching in the latter plant is monopodial, with the leaf more closely related to the side-branch.

Finally, the formation of new plants from adventitious buds which are usually confined to the leaves, may also be included under branching. In accordance with their origin from minute initials, these plants are connected to the parent leaf by a thread, like strand which rapidly dilates and becomes elaborated into the adult type of stele. This method of branching is probably the most recently evolved, but evidently on account of its success as a means of rapid propagation, it has already been established in a very large number of Ferns, in some cases to the detriment of the usual method of reproduction by spores.

Just as the relation of adventitious buds to primary buds is obscure, it would be difficult to relate this latest mode of branching to the usual method, in which the branch always springs from a bud originally laid down at the growing apex of the mother axis.

IV. SUMMARY AND CONCLUSIONS.

The most important direction in which the evolution of the filicinean branch system has progressed is specialization for vegetative propagation. This is only an elaboration of the fundamental idea involved in branching, namely, the amplification of the plant-body. Subsidiary efforts have been made in the direction of (a) epiphytism (*Nephrolepis volubilis*), (b) food or water storage (*Nephrolepis tuberosa*, *Polypodium Brunei*, *Hymenophyllum Ulei* and *H. lineare*.¹)

On taking a bird's-eye view of the branching of Ferns it is observed that the different forms may be arranged in a series beginning with those in which the rhizome divides into two more or less equal branches, and ending with forms in which the proximal part of one of the branches attains a great length and bears either reduced leaves (*Struthiopteris*, etc.) or none at all (*Nephrolepis*). This portion, which has been called a stolon, serves to remove the leafy apex of the branch away from the mother-axis, and to feed it before it establishes an independent root-system. In the extreme case (*Nephrolepis*) the stolon is capable of producing a large number of lateral branches each of which is a potential

¹ Giesenhagen (Berichte, 1909, pp. 331,333) considers the tuberosous bodies in *Hymenophyllum Ulei* and *H. lineare* as insect-galls, but Christ (Geographie der Farne, 1910, p. 99) regards them as water-storing organs.

individual. The local reduction or absence of leaves in the latter group of forms allows of no doubt that the mode of branching they possess is a highly specialized one, and it is most natural to derive it from the dichotomous type illustrated by the former group: the two extremes are connected by an unbroken series of transitions (Fig. 1 C—G, p. 5).

This conclusion is corroborated by a study of the branching of Ferns from the point of view of their vascular anatomy. This affords a series of transitions parallel to the above, while independent grounds are given for the view that the latter ends of these series represent the derivative, the former the primitive condition. For a *résumé* of our knowledge of the vascular relations of branch and stem throughout those Filicales in which branching has been described, the reader is referred to p. 9 and Fig. 1, p. 5.

When the growing apex of a Fern stem divides in preparation for branching, the resulting growing points may either both continue their growth simultaneously, or, as very commonly happens, one of them may almost immediately become dormant, while the other continues its growth in the direction of the original axis. A third possibility is that the dormancy of one of the growing points may be delayed for a variable period of time during which the two have been growing together as in the first case. Of these three conditions the second will be generally admitted to be derived from the first, since it involves a sacrifice of one of the branches for the good of the other; while the third condition may be considered to be intermediate between the other two.

An inquiry into the circumstances under which the different forms of branching occur shows that the dichotomous types correspond to the first of the cases mentioned above, while in the extreme monopodial types the branches arise from buds which become dormant immediately after their origin, when they were still minute. The intermediate types probably correspond with the third case.

There is no necessary parallelism between the evolution of the modes of branching on the one hand, and the evolution of the plants themselves on the other: the two processes must therefore have been independent of each other. Dichotomy still persists among the higher Ferns, while some of the most primitive Ferns (Ophioglossaceæ) show an advanced monopodial type of branching.

The monopodial type of branching has been derived from the dichotomous by a process of retrogressive evolution in the basipetal direction, involving the successive intercalation, at the base of the

branch, of a series of stages, each morphologically less complex than the preceding. This process naturally finds its full illustration in forms with reticulate steles. The basal protostele of the specialized branch is therefore a cœnogenetic feature, not strictly primitive.

The departure from dichotomy was entered upon at a very early period in the history of the Filicales.

We may look upon the simpler organization of the specialized branch in its proximal region as a sudden drop to a morphologically lower and more stable level in consequence of a loss of physiological conditions necessary for the production of the morphologically higher condition. The alleged retrospective nature of traumatic structures may perhaps also be regarded in the same light.

Viewing the group of Filicales as a whole it may be said that the branches do not hold any regular position with respect to the leaves. In those cases where the branches arise in some relation to leaves, this association is, in its evolutionary origin, a secondary phenomenon attributable to possible biological advantages, one of which may be the protection of the young bud. Significant in this connection is the fact that in some plants possessing so-called axillary branches (Hymenophyllaceæ, Ophioglossaceæ) while the stele of the branch is sometimes inserted on that of the main axis independently of the subtending leaf-trace, it is sometimes confluent with the latter at the base. The view here adopted is that in all such cases the portion of the strand common to leaf and branch, conveniently termed by Dr. Scott the "undivided trace," is of a dual nature; we owe this view originally to Dr. Scott, but he rejected it in favour of the foliar view. Professor Bower's description of *Cheiropleuria* strongly supports the former view.

The formation of new individuals from adventitious buds on the leaves is the most recently evolved method of branching in Ferns, but it is difficult to relate it to the usual method, in which the branches always spring from buds laid down at the growing apex of the mother-axis.

My hearty thanks are due to Mr. Tansley, not only for valuable suggestions regarding the literature, but also for much helpful advice and criticism during the preparation of the manuscript of this paper. I have pleasure also in heartily thanking Professor Seward for references to literature.

THE BOTANY SCHOOL, CAMBRIDGE,
December, 1916.

CARBON ASSIMILATION.

A REVIEW OF RECENT WORK ON THE PIGMENTS OF THE
GREEN LEAF AND THE PROCESSES CONNECTED WITH THEM.

BY INGVAR JØRGENSEN AND WALTER STILES.

(Continued from Vol. XV, p. 232).

CHAPTER VI.

Energy Relations in Carbon Assimilation.

A. GENERAL REMARKS.

In the introductory chapter we have referred to the fundamental fact that radiant energy is utilised in carbon assimilation so that compounds of higher energy content are produced from the simpler ones of the surroundings. Beyond this the energy relations of the green leaf are only very imperfectly known, although physical and chemical methods for investigating such energy relations have reached a high degree of development. This aspect of carbon assimilation exhibits perhaps more than any other an unfortunate isolation of effort in research, the various workers on the subject having generally neglected the results obtained by others, both along their own and related lines of investigation.

As in those aspects of the subject we have already dealt with, so here also the complexity of the processes is again evident, and it is difficult to draw definite conclusions. It is possible to measure quantitatively the radiant energy incident on the leaf, and also to measure the amount transmitted. It is, however, by no means easy to determine in what way the energy absorbed is utilised, because we do not know with any approach to completeness how this energy is expended into chemical or electrical energy or heat.

It is generally assumed that the increase in the heat of combustion of the leaf represents that part of the absorbed energy transformed into chemical energy, but it should be pointed out that in so doing, carbon assimilation is taken in its widest possible sense (not carbohydrate assimilation merely) so as to include all the substances formed in the leaf as a result of the photochemical and possible contemporary processes. Thus it is by no means settled as to what extent proteins are formed in the green leaf by photochemical or other chemical actions. In any case an error is introduced if proteins are formed, as their products of combustion cannot be identical with the substances in the leaf from which they are produced, so that their heat of combustion cannot have the same value as the radiant energy used in the leaf in their formation.

We shall first discuss the methods for estimating the amount

of material produced in assimilation and the conclusions which have been drawn as to the energy relations of the green leaf from such determinations, before passing on to a discussion of work in which quantitative measurements of both radiant energy and heats of combustion have been made. Finally, we shall briefly deal with work on the assimilatory power of light of different wave-lengths.

B. QUANTITATIVE ESTIMATION OF CARBON ASSIMILATION

BY MEANS OF THE PRODUCTS.

In an earlier chapter we have dealt with Blackman's and Willstätter's estimation of carbon assimilation. Both these workers employed a method based on that of Kreuzler, in which the intake of carbon dioxide by the leaf is used as a measure of carbon assimilation.¹ The assimilation could also be measured by estimating the increase of carbon content of the leaf, but what is more usually done is to measure the increase of dry weight of the leaf and assume that this is proportional to the increase in carbon content.

Brown and Escombe (1905) in their attempt to determine the energy relations of the leaf, compared the increase of dry matter with the intake of carbon dioxide, but as their results obtained by the two methods were not concordant they came to the conclusion that the dry weight method is untrustworthy. Therefore they only determined the intake of carbon dioxide and estimated the increase in dry matter by calculation. We shall only deal briefly with the extensive researches of Brown and Escombe on this subject, for although they are the first to make quantitative measurements of energy in regard to assimilation and although they clearly indicate the complexity of the energy relations of the leaf, yet the values actually determined by experiment are few and those obtained by calculation are of doubtful value and do not agree with values obtained by direct measurement by other investigators. We have already referred to the divergence between the estimations of the internal leaf temperature made by Brown and Escombe, and the direct temperature measurements made by Blackman and Matthaei.

In order to estimate the dry matter formed, Brown and Escombe multiply the weight of carbon dioxide absorbed by the leaf by a

¹ The same method has been employed by Brown and Escombe (1902). We have not dealt with the very interesting results recorded in this paper in regard to carbon dioxide as a limiting factor, as the results are confirmed by F. F. Blackman's later and more complete work on limiting factors. In order to avoid unnecessary length of this review we have in this matter, as elsewhere, confined our remarks to the more complete account where two researches run parallel.

carbohydrate factor of 0.640. This factor they obtain from the analyses in regard to carbohydrate content of leaves of *Tropaeolum majus* by Brown and Morris as described in the last chapter. Of course Brown and Escombe assume that the ratio between the various carbohydrates remains constant and further that carbon dioxide is used only in the production of carbohydrates. It is true that variations in the ratio of the various carbohydrates will make little difference in the carbohydrate factor, and similarly, the error introduced owing to the probable incorrectness of Brown and Morris's analysis (cf. Chapter V) is likely to be small. On the other hand the error introduced by the assumption that the whole of the carbon dioxide is used in carbohydrate formation is likely to be larger, but on this subject our information is very incomplete. It may be interesting to compare this carbohydrate factor with values obtained by experiment for the ratio between increase in dry weight and carbon dioxide absorbed. The following table is due to Krasheninikov (1901) and although the values are probably not of a very high order of accuracy, they may give some idea of the variations likely to occur.

TABLE XXXVI.

Increase in Dry Weight of Leaves per gram of Carbon dioxide absorbed.

Bamboo	0.60
Cherry Laurel		0.60
Sugar Cane	0.67
Lime	0.74
Tobacco	0.68

Thoday (1909) compared the increase in dry weight with the increase in carbon content of the leaf in the cases of *Helianthus tuberosus* and Cherry Laurel. His results indicate a considerable variation in the ratio of carbon increase to increase of dry weight in leaves of the same species, but in Thoday's experiments a good many factors are not controlled, and it is impossible to say what causes the variation. We should like to emphasize that in all such cases in plant physiological researches where it is sought to determine the absolute value of a quantity, it is absolutely imperative to determine the probable error of the experiment. This, as far as we know, has not been done in any single instance in work on carbon assimilation.

In regard to the direct determination of the products of assimilation, the principle of this method was first exposed by Sachs

in his paper "Ein Beitrag zur Kenntniss der Ernahrungsthätigkeit der Blätter" published in 1884. Sachs' method is well-known. The dry weight of unit area of one half of a leaf measured at the beginning of an experiment is compared with the dry weight per unit area of the other half of the leaf after its exposure to the required conditions. The difference of the two values is regarded as the weight of products which have accumulated in unit area of the leaf during the experiment. As Sachs found a greater increase in dry weight in detached leaves than in leaves still attached to the plant, he assumed that in the latter case translocation of the products away from the leaf takes place concurrently with assimilation. To obtain the true value for assimilation in attached leaves, Sachs therefore added the loss in dry weight of leaves during the night to the increase in dry weight of the same area during the same time during the day.

Brown and Escombe pointed out that Sachs obtained much higher values for assimilation by his half leaf method than they obtained by direct determination of the carbon dioxide absorbed. They therefore carried out a series of experiments in which the assimilation of the same leaves were measured by both methods. The following table gives the results they obtained for *Catalpa bignonioides*. The results in the last column are obtained by the use of the carbohydrate factor 0.64 already referred to.

TABLE XXXVII.

Comparison of the Values obtained for Assimilation of Leaves of Catalpa bignonioides by the Half-Leaf Method and by measuring the Intake of Carbon dioxide.

Experiment.	Increase in Dry Wt. per sq. decimetre per hour mg. (observed).	CO ₂ absorbed per sq. decimetre per hour, ccs.	Carbohydrate formed per sq. dec. per hour, mg. (calculated).
1	9.83	1.41	1.76
2	7.14	1.43	1.79
3	2.60	2.35	2.94
4	7.22	2.33	2.92
Mean	6.69		2.35

It will be observed that the divergence between the results obtained by the two methods is much larger than can be accounted for by experimental error or error in the estimation of the carbohydrate factor. Brown and Escombe attribute this divergence to three sources of serious error to which the half leaf method is liable. These are—

1. Possible changes after assimilation in the power of retention of water by the colloids of the cell contents when these are dried at 100°C.

2. Differences due to lack of symmetry between the two halves of the leaf in regard to venation and thickness.

3. Alterations of area of the leaf as a result of insolation. Thus if a leaf suffered shrinkage during insolation so that its area as measured afterwards was less than at the beginning of the experiment, the dry weight of unit area would be correspondingly increased. Consequently the values found for the increase in dry weight of unit area would be larger than the true values as, of course, the initial dry weight is measured before insolation on an unshrunk half leaf.

1. The possible error due to changes of composition during insolation which might produce a different water retaining capacity was investigated by Thoday (1909), who measured both the dry weight and carbon content of the experimental and control half leaves and so calculated both the gain in dry weight and of carbon per unit area. Thoday concludes that the correspondence between the increase in dry weight and the starch equivalent of the gain in carbon is sufficiently close to make it clear that fixation of water cannot play an appreciable part in determining the dry weight increase. However, as the starch equivalent of the gain in carbon found in Thoday's experiments varied from 20% less to 40% (and in one extreme case 90%) more than the actual increase in dry weight of the same leaf, it is not clear why Thoday should come to this conclusion from his results. We hesitate therefore to accept Thoday's own opinion that his results indicate that the dry weight method is "not vitiated by any large indeterminable errors such as would arise if varying quantities of water were retained by the colloids of the leaf after drying it at 100°C." The numbers show indeed that changes in composition of the leaf during assimilation will not account for the whole of the discrepancy between the two methods as observed by Brown and Escombe, but they tell us nothing as to whether such change is negligible or not.

2. Brown and Escombe made a number of determinations of the degree of symmetry of the two halves of various leaves by measuring the two halves separately with a planimeter and then drying them to a constant weight. The dry weight per square decimetre of the two halves was calculated and the percentage difference between the dry weight per unit area of the two sides of

the leaf calculated. These differences are summarised in the following table.

TABLE XXXVIII.

Difference in Dry Weight per Unit Area of Opposite Sides of Leaves due to Differences in Symmetry (Brown and Escombe).

Species.			Difference in Dry Weight, Per Cent.		
Catalpa bignonioides	3.9
"	"	4.3
"	"	2.3
"	"	5.7
"	"	0.7
Catalpa purpurea	2.3
Catalpa Bungei	1.3
"	"	2.2
Tropæolum majus	0.3
Polygonum Weyrichii	1.1

Similar differences were found by Thoday for some other species and he concludes with Brown and Escombe that this source of error is inherent in the method. He points out that the error arising from this cause may be reduced by using parts of leaves free from big veins instead of whole half leaves. Thus with *Paulownia imperialis* the average percentage difference of four pairs of measurements was 1.4% when the veins were avoided and the average percentage difference of two pairs of measurements was 5.95% when the veins were included.

3. The third source of error suggested by Brown and Escombe is that due to change in area of the leaf during insolation. These investigators measured the area of leaves of *Catalpa bignonioides* before and after insolation and found resulting alterations in area from an increase of 0.14% to a decrease of 3.12%. According to Thoday, leaves of *Helianthus annuus* often diminish in area by more than 5% between early morning and midday if the meteorological conditions are such as to favour rapid transpiration of water.

From such data Brown and Escombe calculate the order of magnitude of the error likely to arise in Sachs' half leaf method. It would thus be quite probable for the error in determination of the dry weight of a half leaf to equal 2%. In such a case Brown and Morris show that with a leaf having a dry weight of 0.5 gm. per sq. decimetre assimilating 0.002 gm. carbohydrate per sq.

decimetre per hour the error in the increase in dry weight obtained by the half leaf method in an experiment lasting 5 hours would be as much as 100%, whereas the error in the results obtained by measuring the carbon dioxide absorption would amount to no more than 2%. They therefore reject Sachs' method as quite untrustworthy.

As the dry weight method, if it could be made sufficiently accurate, would have its uses we agree with Thoday "that it should not lightly be abandoned." Thoday makes some useful suggestions in regard to decreasing the inaccuracy of the method, but he does not furnish data which enable one to determine the degree of accuracy obtainable when all suggested precautions are taken. It appears to us that the only way of finding this is to make a number of such estimations and determine the probable error of the mean result.

C. THE QUANTITATIVE DETERMINATION OF THE HEAT OF COMBUSTION OF THE PRODUCTS OF ASSIMILATION.

Although the measurement of heats of combustion offers no particular difficulties, very few such measurements have been made in plant physiology. Brown and Escombe assume that the heat of combustion of the products of assimilation is the same as that of glucose, but this assumption is not justified by the values obtained by experiment for the heat of combustion of one gram of material produced in assimilation. It will be seen however from the heats of combustion of various substances recorded in the accompanying table, that measurements of the actual heats of combustion of the products of assimilation might afford helpful information as to the relative proportion of the different products.

TABLE XXXIX.

Heats of Combustion in Gram-Calories of Various Substances.

Substance				Heat of Combustion per gram.
Ethyl Alcohol	7.18×10^3
Glucose	3.76×10^3
Sucrose	3.99×10^3
Dextrin	4.1×10^3
Starch	4.1×10^3
Cellulose	4.2×10^3
Leucin	6.5×10^3
Vitellin	5.7×10^3
Linseed Oil	9.47×10^3
Olive Oil	9.51×10^3

Actual determinations of the heat of combustion of the material produced in assimilation have been made by Krasheninikov (1901) and by Puriewitsch (1914). They measured the increase in dry weight per unit area per hour by Sachs' dry weight method and also the increase in the heat of combustion per unit area per hour. The increase in heat of combustion per unit increase in dry weight gives the heat of combustion per gram of the products of assimilation. Krasheninikov obtained an average value for this of 4.4×10^3 gram-calories. From Puriewitsch's data we have calculated the values for different species set out in the following table. It will be observed that the values agree well with the number obtained by Krasheninikov, but not with the value assumed by Brown and Escombe (3.76×10^3).

TABLE XL.
Heats of Combustion of the Products of Assimilation.

Species.	Increase in Dry Wt. per sq. metre per hr., gm.	Increase in Heat of Combustion per sq. cm. per hr.	Heat of Combustion of Product of Ass. in gm.-cal. per gram.
Acer platinoides	1.2	0.526	4.4×10^3
Polygonum sacchalinese	2.7	1.41	5.2×10^3
" "	2.0	0.903	4.5×10^3

D. THE QUANTITATIVE MEASUREMENT OF THE RADIANT ENERGY INCIDENT ON THE LEAF AND THE UTILISATION OF THIS ENERGY.

A full discussion of the methods used and principles involved in the measurement of radiant energy would be out of place here. The instruments generally employed are of four kinds, the thermopile, the bolometer, the radiometer and radiomicrometer. For a description of these instruments the reader is referred to physical text books, and for a more complete discussion on the relative merits of the various methods, to Kayser's *Spectroscopie*, Baly's *Spectroscopy*, and Coblentz's "Instruments and Methods used in Radiometry" (1908). Generally speaking, the radiant energy is absorbed and transformed to heat in the measuring instrument, and thus a measure of the total energy obtained, but if by a suitable method a spectrum of the source of light is produced, the same method can be used for measuring the distribution of energy in the different parts of the spectrum.

Although numerous measurements of the radiant energy of the sun have been made by astrophysicists, yet such quantitative measurements of radiant energy as have been made in plant physiological experiments are inadequate, and in plant ecological studies where light may be an all-important factor, such measurements have not even been attempted.¹

Detlefsen (1888) appears to be the first to attempt energy measurements in regard to problems connected with carbon assimilation. He showed that more energy is used when the leaf is supplied with an atmosphere containing carbon dioxide, than in an atmosphere devoid of this gas. Similar determinations have been made by Mayer (1893) and Ursprung (1903) by the use of a thermopile, but Brown and Escombe (1905) were the first to make an extensive series of measurements of radiant energy in connection with plant physiological problems.

Brown and Escombe measured the intensity of radiation on the leaf by means of a pair of differential platinum thermometers, one bright and the other black, as devised by Callendar (1898). The instrument was rendered self-recording by connecting it with a Callendar's recorder (1899).

The characteristic feature of Brown and Escombe's work on the energy relations of the leaf is that they assume there are certain fundamental properties of the leaf in regard to energy, and they attempt to determine certain physical quantities, such as coefficient of absorption and emissivity, which they regard as constant for all conditions of experiment. But even a superficial consideration is sufficient to tell us that this cannot be the case and the performance of a larger number of experiments would probably have shown these authors what range of variations were likely to be obtained. Thus Puriewitsch insists that the absorption of energy depends on the concentration of carbon dioxide; his results are given in the table overleaf.

The beauty of Brown and Escombe's work lies not in the reliability of the results obtained by measurement or calculation, but in the fact that they are the first, and up to now, the only investigators who have attempted to obtain a complete balance sheet for the leaf in regard to energy.

¹ We do not discuss here the elaborate work of Wiesner (1907), which although interesting in its conceptions of light in respect of plant physiological and ecological problems, does not render much help to the problems under review on account of the inadequate method of energy intensity measurement by means of photographic paper. On the other hand many of the observations recorded may become useful as a basis for future observations.

TABLE XLI.

*The Absorption of Radiant Energy by the Leaf in the Presence
and in the Absence of Carbon Dioxide (Puriewitsch).*

Species.	Date.	Duration of Experiment.	CO ₂ in air, per cent.	Ratio of Energy transmitted in presence of CO ₂ to that transmitted in absence of CO ₂ , per cent.	Ratio of Excess of Energy absorbed by the leaf in presence of CO ₂ to that transmitted in absence of CO ₂ , per cent.
Aristolochia Sipho	27 May 1910	11.25 a.m.-12.43 p.m.	1.2	95.0	5.0
		1.1—2.8 p.m.	„	90.4*	9.6
„ „	„ „	11.25 a.m.-12.43 p.m.	„	92.2	7.8
		1.1—2.8 p.m.	„	95.4*	4.6
Catalpa speciosa	1 June 1910	11.55 a.m.-1.0 p.m.	0.7	99.0	1.0
Acer platanoides	2 June 1910	11.29 a.m.-1.14 p.m.	1.7	98.3	1.7

*The values marked with an asterisk were obtained by means of a Rubens thermopile, the remainder by means of the Bolometer.

Brown and Escombe suppose that the total radiant energy falling on the leaf is used in the following ways ;

- (1) in assimilation,
- (2) in transpiration,
- (3) by transmission through the leaf,

(4) by thermal emission (if the leaf temperature is higher than that of its surroundings, as it usually is, this is positive, but if lower the thermal emission is negative, that is, the leaf gains energy from its surroundings).

We have indicated earlier in this chapter that Brown and Escombe estimated the assimilation by calculating the increase in dry weight from the intake of carbon dioxide and the assumed heat of combustion of the products. Thus, in this first determination, two assumptions are made, the accuracy of which are not confirmed by measurement. One of these is the assumption that 1 gram of carbon dioxide absorbed is equivalent to 0.64 gram of dry matter ; the other that the heat of combustion of the products is 3.76×10^3 gram-calories.

The transpiration was determined by weight, and the energy used in transpiration calculated from the heat of vaporisation of water at the particular temperature.

The energy transmitted was calculated from the coefficient of absorption of the leaf, which was found in the following manner. A day of bright sunshine was selected and the intensity of radiation

of sunlight measured. The leaf was interposed above the coils of the instrument for a few minutes and the intensity of radiation again measured. The leaf was then withdrawn when the value of the full intensity of radiation was again recorded on the drum of the self recorder. The ratio of the middle reading to the mean of the first and third readings gives the coefficient of transmission, and the difference between unity and the coefficient of transmission is the coefficient of absorption. The following table shows the coefficients of absorption and transmission found by Brown and Escombe for various species.

TABLE XLII.

Coefficients of Absorption and Transmission of Radiant Energy of Sunlight.

Species.	Coefficient. of Absorption.	Coefficient of Transmission.
<i>Helianthus annuus</i>	0·686	0·314
<i>Polygonum Weyrichii</i>	0·647	0·353
„ <i>Sacchalinese</i>	0·691	0·309
<i>Petasites officinalis</i>	0·728	0·272
<i>Silphium terebinthaccum</i>	0·699	0·301
<i>Arctium majus</i>	0·728	0·272
<i>Verbascum olympicum</i>	0·758	0·242
<i>Senecio grandifolius</i>	0·774	0·226

No considerable difference in the coefficient of absorption was found between leaves of the same species of different ages.

Of course, in these determinations the part of the energy reflected from the surface of the leaf is neglected. Brown and Escombe regard the reflected energy as forming a very small fraction of the total incident energy, but having regard to the information available from pure physics it is unlikely to be negligible, as a black cloth, for instance, may reflect 1% of the radiant energy incident upon it.

The difference between the total incident energy absorbed on the one hand, and that used in assimilation and transpiration on the other hand, gives that part of the energy lost by re-radiation, conduction and convection, *i.e.*, that lost by emission.

The following numbers show the results of a typical experiment.

Tropæolum majus, September 11th, 1900.

Leaves in sunlight under canvas screen.

Duration of experiment 4·8 hours.

Assimilation of CO ₂ per sq. decimetre per hour ...	1·210 c.c.
Transpiration " " " " ...	0·1340 gm.
Solar radiation incident on leaf per sq. cm. per min.	0·1282 gm.-cal.
Coefficient of absorption	0·700
Solar radiation absorbed by the leaf	0·0897 gm.-cal.
Energy expended in assimilation	0·0010 " "
" " " transpiration	0·0132 " "
Total Energy used for internal work	0·0142 " "
Energy lost by re-radiation and convection ...	0·0755 " "

In the following table we give the summary of the energy relations as estimated by Brown and Escombe in five such experiments on *Polygonum Weyrichii*.

TABLE XLIII.

Mode of Disposal of Energy by the Leaf of Polygonum Weyrichii.

Total Energy Received 100.

Experiment.	Energy used in Assimilation.	Energy used in Transpiration.	Total Energy expended in Internal work.	Energy lost by Transmission.	Energy lost by Re-radiation and Air Convection.
1	0·42	9·67	10·09	35·31	54·60
2	1·59	53·60	55·19	35·30	9·51
3	1·66	57·01	58·67	35·32	6·01
4	1·32	35·64	36·98	35·28	27·76
5	0·49	52·72	53·21	35·30	11·49

In order to indicate the conditions of experiment, we give below (Table XLIV) the actual experimental data of these five experiments as recorded by Brown and Escombe.

We do not intend to analyse in detail the results obtained by Brown and Escombe, as for reasons already given, we consider that at present it is not possible to obtain absolute values of the energy used in different processes. More light must be shed on the complexity and influence of the various factors before such numbers as Brown and Escombe's can be discussed with profit.

How much importance can be attached to such calculated values is indicated by the estimations of leaf temperature by Brown and Escombe. To obtain this they start with the assumption that

TABLE XLIV.
Experiments on Leaves of Polygonum Weyrichii under Various Conditions of Insolation.

Experiment.	Date.	Conditions of Experiment.	Assimilation in c.cs. CO ₂ per sq. decimetre per hr.	Transpiration in gms. per sq. decimetre per hr.	Solar Radiation incident on Leaf.	Solar Radiation absorbed by Leaf.	Energy used in Assimilation.	Energy used in Transpiration.	Total Energy used in Internal Work.	Energy lost by Re-radiation and Air convection.
					In gm.-calories per sq. cm. per minute.					
1	June 29, 1900	Intermittent sunlight without any screen.	3.20	0.599	0.6120	0.3959	0.0026	0.0592	0.0618	0.3341
2	June 19, 1900	Full sunshine. Leaves under thin canvas screen.	3.758	1.054	0.1942	0.1256	0.0031	0.1041	0.1072	0.0184
3	June 22, 1900	Intermittent sunlight. Leaves under thin canvas screen.	3.058	0.868	0.1503	0.0972	0.0025	0.0857	0.0882	0.0090
4	July 3, 1900	Intermittent sunshine with some showers. Leaves under canvas screen.	2.271	0.517	0.1431	0.0926	0.0019	0.0510	0.0529	0.0397
5	July 11, 1900	Hot cloudless day. Leaves under thin canvas screen.	1.479	1.291	0.2418	0.1565	0.0012	0.1275	0.1287	0.0278

if a leaf is transpiring in the dark, its temperature will fall until the energy used in transpiration is equal to that which it receives from its surroundings in the same time. In some experiments made by Brown and Wilson (1905) it was found by measuring the temperature of leaves and the loss of water by transpiration, that when there is a temperature difference of 1°C between the leaf and its surroundings, the leaf of *Tropæolum majus*, for example, receives or loses, as the case may be, 0.01427 calories per square centimetre per minute in still air. This is the thermal emissivity. In Brown and Escombe's experiments the total energy lost by radiation is obtained by difference, and the division of this number by the thermal emissivity calculated by Brown and Wilson is supposed to give the difference in temperature between the leaf and its surroundings. As the temperature of the latter is measured, that of the leaf is at once deduced.

We do not propose to discuss Brown and Wilson's method of finding the thermal emissivity, as the method of estimating the leaf temperature is crude, and other factors which may be important, such as respiration and temperature, are regarded as negligible. It is only to be expected that the temperatures so estimated should be far removed from the real temperatures. As Blackman and Matthaei (1905) point out, the temperatures given in Brown and Escombe's tables "for leaves in the sun in the open air are never more than 2°C above the shade temperature of the air, while our few direct measurements with cherry-laurel leaves, brilliantly insolated, indicated 7° to 16°C above the thermometer in the shade." We may add that differences similar to those observed by Blackman and Matthaei and of even greater magnitude have been observed by various workers, notably by Askenasy (1875), Ewart (1897) and Stahl (1909).

Brown and Escombe's results show that only a small proportion of the energy absorbed by the leaf is used in carbon assimilation, but that the actual percentage used for this purpose is a very variable quantity. This conclusion has also been reached by Puriewitsch (1914), who measured the total radiant energy incident on the leaf by means of the bolometer (see Kurlbaum, 1894). The energy used in assimilation was obtained in some cases by direct measurement of the increase of the heat of combustion per unit area of the leaf as described in the previous section of this chapter. Unfortunately, only a few such determinations were made, and the values for the remaining experiments calculated from them.

We may quote the results of a typical experiment of Puriewitsch.

Two leaves of *Acer platanoides* were used. The intensity of radiation incident on the leaf was measured every 10 minutes during the experiment which lasted 6 hours, and the total energy calculated.

The increase in dry weight was obtained by the half-leaf method.

The following results were obtained:—

	Before Insolation.	After Insolation.
Area of half leaf	316.6 sq. cm.	316.8 sq. cm.
Dry weight of half leaf ...	1.2494 gm.	1.3952 gm.
Dry weight per sq. cm. ...	0.0039 gm.	0.0044 gm.
Heat of combustion of 1 gm. dry weight	4300.21 gm.-cal.	4313.46 gm.-cal.
Heat of combustion per sq. cm.	16.770 „	18.978 „
Increase of heat of combustion after insolation per sq. cm.—	2.208 gm.-cal.	

Total energy incident on leaf per sq. cm. 361.03 gm.-cal.

Quantity of radiant energy used in assimilation 0.6%.

As we have stated before, conclusions in regard to the relation between the intensity of the radiant energy and that part of it used in assimilation, cannot be drawn until our information in regard to the various factors is considerably enlarged. It can, however, be concluded that with high light intensities only a small part of the incident radiant energy is utilised for assimilation. The lowest value, for instance, obtained by Puriewitsch was 0.6% for an average light intensity of 1.003 gm.-calories per minute. But it will clearly be seen from the table below that other factors besides light intensity are operating.

Brown and Escombe's figures exhibit similar variations in the percentage of sun energy utilised in assimilation, but on the whole they are lower. This difference is easily accounted for by the different method employed in measuring the assimilation. Brown and Escombe, as we have shown, used the intake of carbon dioxide in conjunction with a carbohydrate factor and an estimated value of the heat of combustion in order to obtain a measure of the energy used in assimilation. Their method is likely to yield more uniform though perhaps not more accurate results than the half leaf method employed by Puriewitsch, who did not attempt to correct any of the sources of error of the method, which it might

TABLE XLV.

Percentage of Radiant Energy Incident on the Leaf used in Assimilation (Data from Puriewitsch).

Species.	Date.	Duration of Experiment in hrs. & min.	Total Incident Energy per sq. cm. in gm.-cal.	Increase in heat of combustion per sq. cm. in g.-cal.	Intensity of Radiant Energy in gm.-cal. per sq. cm. per min.	Percentage of sun energy used in Assimilation.
<i>Acer platanoides</i>	30 May, 1912	6.0	361.03	2.208	1.003	0.6
" "	2 June, "	5.0	162.59	1.332	0.542	0.81
" "	13 " "	6.0	240.33	6.508	0.667	2.7
" "	19 " "	5.0	202.20	2.630*	0.674	1.3
<i>Helianthus annuus</i>	11 " "	4.30	132.48	5.977	0.454	4.5
<i>Polygonum sacchalinese</i>	31 May, "	1.20	70.85	5.509	0.885	7.7
" "	3 June, "	3.0	122.33	5.076	0.679	4.1
" "	16 " "	1.50	97.62	2.585*	0.887	2.6
" "	17 " "	2.20	123.18	4.656	0.880	3.7
" "	21 " "	5.0	136.81	1.540	0.456	1.1
" "	23 " "	5.0	177.00	4.514*	0.590	2.5
<i>Saxifraga cordifolia</i>	6 " "	2.20	68.16	3.450	0.487	5.0

*The values marked with an asterisk were actually observed. The remaining values in this column were obtained by calculation.

have been possible to correct.

We may now attempt to correlate the results of Brown and Escombe and of Puriewitsch with those of Blackman on light as a factor in assimilation. It will be recalled that on Blackman's view of limiting factors, if we commence with a very low light intensity, increase in light (radiant energy) will result in a proportionate increase in assimilation until some other factor, such as carbon dioxide supply, is limiting the rate. The curve connecting the light intensity and the rate of assimilation will be of the form already shown in Fig. 5. As regards the *proportion* of the radiant energy used in assimilation, this should remain constant on Blackman's view so long as light is the limiting factor, for the rate of assimilation, and consequently the energy used for it, is directly proportional to the intensity of the light. But when the light is increased so that some other factor is limiting the rate of assimilation, then if that factor remains constant, increase in light intensity will result in a decrease in the percentage of radiant energy used in assimilation.

In many of Brown and Escombe's experiments the intensity of illumination is roughly inversely proportional to the proportion of the energy used in assimilation, but this relation is not by any means

exact or even approximate. It suggests, however, that in some of Brown and Escombe's experiments at any rate, radiant energy was in excess, and some other factor was limiting the rate of assimilation. In a set of experiments they performed in which the proportion of the full radiant energy of the sun utilised in assimilation was compared with that proportion of it so utilised when it was reduced to a fraction of the full energy by means of rotating sectors placed above the leaf, it was always found that reducing the intensity of illumination increased the proportion of energy used in assimilation. This is exactly what one would expect, as in the experimental arrangement of Brown and Escombe, the full intensity of radiant energy falling on the leaf would be likely to be in excess of that required for the carbon dioxide supply.

Similarly, in Puriewitsch's experiments, although unfortunately no data whatever are given in regard to temperature, it seems likely that the radiant energy was not limiting the rate of assimilation. The carbon dioxide supply was low, namely that of the atmosphere. The intensity of radiation was, on the other hand, in all cases moderately high. Under these conditions we should expect that carbon dioxide supply would be the limiting factor and that the variations in the total radiant energy in the different experiments would be without influence on the rate of assimilation. Consequently we should expect that the proportion of the sun energy used in assimilation would vary inversely with the intensity of illumination. Such, however, is not by any means the case, and it is clear we must look for other factors of which no data are given, to explain the results obtained. Puriewitsch does indeed point out that his numbers show that the rate of assimilation falls off with time (cf. Blackman's time factor) but this will not explain his results completely. We have here a particular instance of that lack of correlation of effort to which we have referred in the first section of this chapter, for if Puriewitsch had taken cognizance of Blackman's researches, his experiments might have yielded results of much greater significance. It is only fair to Puriewitsch to point out that he regards his experiments as preliminary.

E. ASSIMILATION IN RELATION TO RADIANT ENERGY OF DIFFERENT WAVE-LENGTHS.

On this subject no satisfactory work has so far been performed, although it has been a favourite subject for investigation for more than a century. On the one hand in no case is the method employed for the

measurement of energy satisfactory, and on the other hand the methods used for measuring assimilation are very crude. Also the fundamental aspects of the problem seem to have escaped the notice of most investigators, in spite of its vital importance. Having regard to the present state of our knowledge concerning radiant energy derived from pure physics, and to what we now know of the processes of carbon assimilation, it ought to be possible to attack the problems profitably.¹

Concerning the subject with which we deal in this section, there exists a very voluminous literature, to which undue importance is generally given in text-books.

The earliest investigators, as for instance, Senebier (1785) and Dumas (1824) supposed that the blue-violet rays were of most importance in assimilation. Daubeny (1836) and Draper (1844) as well as Sachs (1864) and Pfeffer (1871) were of the opinion that the yellow rays were those utilised in assimilation. Lommel in 1871 suggested that the rays most strongly absorbed by chlorophyll, namely those between the B and C lines in the red part of the spectrum, were those most active in assimilation.

Attempts have also been made by Timiriazeff (1877, 1885), Reinke (1884) and Engelmann (1882, 1884) to discover in which part of the spectrum assimilation takes place. They all agree that maximum assimilation takes place in the red part of the spectrum, although they differ as to the exact position. Engelmann, using the most sensitive, though not necessarily the most accurate method, obtains a secondary maximum in the blue-violet end of the spectrum. Timiriazeff and Richter (1902) appear to be aware of the fact that smaller assimilation in the blue part of the spectrum of sunlight may be explained by the less intensity of radiation in that region. Richter and others contend that the assimilation depends only on the energy of the absorbed light and not on the wave length.

The conditions in all these experiments were such that discussion of them is not justified; we mention them here mainly on account of their historical interest.

It does not appear from all this earlier work whether the assimilation would be the same if a leaf were exposed to red or to

¹ We are confronted with the following problems:—

1. The intensities and relative proportions of the different frequencies of radiation incident on the leaf. If sunlight is concerned, astrophysical and meteorological factors are of considerable influence here, but a discussion of this aspect of radiation is outside the scope of this review.

2. The relative absorption by the leaf of radiation of different frequency.

3. The relative proportion of absorbed energy of any particular frequency which is used in assimilation.

blue light of the same energy. This problem has been attacked by Kniep and Minder (1909). They used a Rubens' thermopile for the measurement of radiant energy. Light of different colours were obtained by the use of different filters.

For red a glass filter was used which let through light of wave lengths $620\mu\mu$ —infra red, and a little light of wave length $608\mu\mu$. The coefficients of transmission given for different wave lengths were—

Wave Length.				Coefficient of Transmission.
$\mu\mu$				
644	0.846
578	0.00056
546	0.000057
509	0.000

The blue filter let through light of wave lengths $523.8\mu\mu$ —ultra violet. The coefficients of transmission given for different wave lengths were—

Wave Length.				Coefficient of Transmission.
$\mu\mu$				
546	0.00
509	0.0109
480	0.177
436	0.455
405	0.395
384	0.267
361	0.078
340	0.010
332	0.000

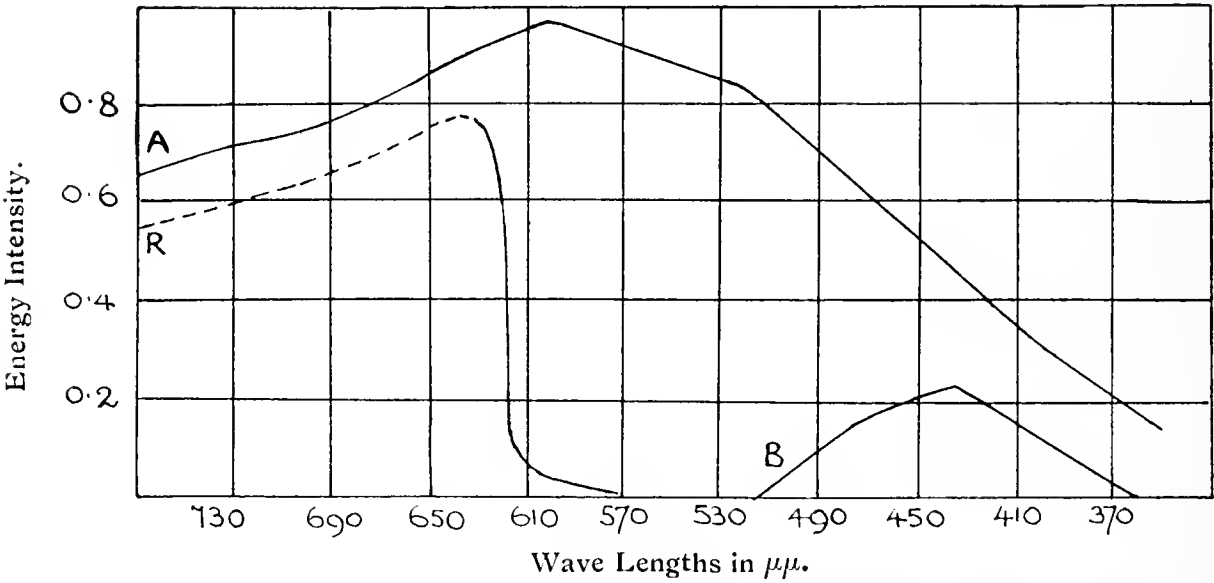


FIG. 18. Energy Intensity in the Normal Spectrum of direct Sunlight after Langley (Curve A), compared with the Energy Intensity in the Spectra of Sunlight after its passage through Red (Curve R) and Blue (Curve B) Filters. The broken part of Curve R is assumed. (After Kniep and Minder).

As a green filter was used a solution obtained by mixing a solution of potassium monochromate with ammonical copper oxide. This solution let through light of wave lengths between $512\mu\mu$ and $524\mu\mu$. No quantitative data were obtained in regard to the coefficients of transmission. From those coefficients of transmission measured and from the curve of distribution of energy in the spectrum of the source of light it is possible to construct curves showing the distribution of energy in the various regions of the spectra of the light let through the filters. So, for instance, the distribution of energy in the solar spectrum has been examined by Langley (1882) and Fig. 18 shows the distribution of energy in the solar spectrum and the distribution of energy in the light let through Kniep and Minder's red and blue filters.

Kniep and Minder only performed their experiments on cloudless days at Naples between 11 a.m. and 2.30 p.m. when the intensity of the light and the distribution of energy in the spectrum¹ remained moderately constant. Heat rays were excluded by the use of screens of distilled water.

It is to be regretted that these authors, after having realised the essential facts of energy distribution in the spectrum and after introducing reliable methods, should render their experiments ineffective by using a method for measuring carbon assimilation which is one of the most unreliable. This method, which consists in measuring the rate at which bubbles of gas are given off by an assimilating submerged water plant,² has recently formed the subject of an investigation by Kniep himself (1915), who shows how many and serious are the sources of error in it. In order to employ this method, Kniep and Minder had to reduce the intensity of radiation by a series of screens of different substances: water, copper sulphate, potassium dichromate, more screens being used for the red than the blue in order to bring the intensity of the radiation to the same value in the two cases. By doing this, of course they necessarily alter the distribution of energy, and the values obtained for transmission and distribution of energy to which we have already referred, have not much bearing on the actual experiments.

¹ The relative intensity of the light of the blue part of the spectrum is very small in the morning, increases towards mid-day, and falls off again in the evening.

² This method, generally known as the "bubbling method," was due, like so much in plant physiology, to Sachs. Accounts of researches in which it was used are to be found in the works of, *e.g.*, Pfeffer (1897), Reinke (1883, 1884), Pantanelli (1903) and Treboux (1903).

The conclusion they draw from their experiments is that blue and red light of the same intensity produce the same assimilation. Green light is incapable of producing assimilation. The absolute intensity of energy incident on their plants is of the order of 0.005 gm.-calories per sq. centimetre per minute, and although it is likely that with this low energy intensity, light is a limiting factor, yet it cannot be assumed that this is so. Kniep and Minder appear to be unaware of Blackman's work on limiting factors, and they give no data relating to factors other than light intensity. It would, therefore, be impossible to draw any valid conclusions from their results, even if their experimental method were beyond criticism.

Investigations along another line have been made by attempting to measure that part of the total energy absorbed by the leaf, which is actually absorbed by the chlorophyll. The experiments of Timiriacheff (1903), in which the absorption of radiant energy by alcoholic extracts of leaves was taken as a measure of the absorption of light by chlorophyll, are clearly of little value, as his leaf extracts would contain far less chlorophyll than impurities. Also the state of aggregation of chlorophyll and its distribution in the leaf are different from those in an alcoholic extract.

Again, the isolated experiments of Brown and Escombe (1905) in which the absorption of radiant energy by the white and green portions of a leaf of *Negundo aceroides* was compared and the difference between the two values attributed to the chlorophyll, is not to be regarded as providing any definite evidence, for it is unfair to assume that the conditions in green and albino parts of a leaf are identical except for the presence of the pigment, and moreover, the considerations we have already put forward in regard to Brown and Escombe's measurements of coefficients of absorption hold equally well here.

Nevertheless, in a comparatively recent publication, Weigert (1911) has accepted Brown and Escombe's result for the *Negundo* leaf, and applied it to work out the efficiency of the assimilatory system for another species. Brown and Escombe had found that in one of their experiments on the energy relations of the leaf, an intensity radiation of 0.5 gm.-calories per sq. centimetre per minute could be reduced to $\frac{1}{12}$ of this amount without diminishing the rate of assimilation; with further reduction of light intensity this became the limiting factor. They estimated the energy used for assimilation at 0.0017 gm.-calories per sq. centimetre per minute *i.e.*, 4.1% of the total incident energy. Now these workers found

in their experiment with the *Negundo* leaf that 4·2% of the total incident energy is absorbed by the chlorophyll. These incidental numbers have been given some importance by Weigert, who assumes from them that $\frac{4\cdot1}{4\cdot2}$ or 98% is the efficiency of the assimilatory system. This result is indeed, as Weigert himself admits, surprising, and he regards the plant as the most ideal photochemical machine which could possibly exist. Presumably Weigert would have been still more surprised if he had chosen a value from another of Brown and Escombe's experiments in which 4·48% of the total energy received by the leaf was used in assimilation. This by his method would have given an efficiency of $\frac{4\cdot48}{4\cdot2}$ or 107%.

But without this absurdity it is clear that numbers obtained from such data are completely valueless.

(To be concluded).

JOHN WILLIAM HART.

ON September 15th, 1916, we lost an ardent student of Nature in the person of John W. Hart, B.Sc., Corporal in the 15th Batt. London Regt. Wounded in the attack on High Wood during the first wave of advance on the Somme, he crawled to the succour of a comrade, but both perished in the heat of battle. It had been his desire to go through the war as a private, but influenced by other considerations, he was, at the time of his death, about to accept a commission.

John Hart was a "son of the soil" in the truest sense, that of filial affection, which appears even in letters written almost under fire, in which he speaks of "such a longing to be amongst the flowers and springing plants—to dabble with seeds and soils."¹ Nature for him included the "stones" also, and he soon became known at the front for his pursuit of fossils, a task that must have cost him many an ache, as one realises in handling—in the light of his own list of compulsory impedimenta—a particular fine specimen of *Sigillaria*, brought home by him in the spring. His enthusiasm spread to his comrades, and he says: "It is really amusing how whenever we are on a fatigue having anything to do with the slag refuse from the mines, I get inundated with

fossils or supposed fossils." When on leave in April, 1916, he remarked regretfully that many, alas, gradually crumbled in his sac after weeks of marching.

His early experience was of farming and cultivation in Canada and at home, and to this invaluable training he later added scientific studies, resulting in the Horticultural Diploma at Reading, and in the London University (War) Degree. The knowledge so gained he applied to the organisation of rural training in which he proved a very stimulating teacher.

To his diversified training he no doubt owed to some extent this diversified interests and acute observation. In his war wanderings he marks down a wood for future ecological survey, and the flora of the trench is evidently always a matter of interest. Thus in June, 1915, he writes: "While passing up the trench—one of a fatigue party—I have noticed a number of interesting plants; in one place a well grown plant of the wild blue Larkspur, which I have not seen wild before, in another a pretty lilac Composite, possibly a species of *Inula* and distinct from a darker one which I spotted among Poppies in front of the trench in which we were last situated—in front and therefore inaccessible!"

The Poppies intrigued both the artist and the botanist in John Hart, for he says: "The scene in front of the trenches beggars description . . . but the feature I shall never forget, as forming such a contrast to the evidences of war, was the colouring . . . here a patch peopled by poppies glowed in the sun with a wonderful red. Yonder another square patch (cornflowers) perfectly delineated, seemed to reflect the blue of the sky; the rest of the ground was divided into such patches, some red, some blue, some purple, from an admixture of the two plants in varying but constant proportions. In one place the white chalk of the German parapet was backed by a strip of pure scarlet, whilst in front the trench was lined with a deep blue line—a perfect tricolor—surely a presage of victory." And again, "The poppies here are immensely interesting, many species, varieties, and perhaps hybrids, occurring. I spent this morning sketching and describing some of these. One distinct and interesting type of procumbent habit, has a small flower, petals equal and distant, fruit 3—4 times as long as broad, the flower and fruit being suggestively cruciferous."

But the war did not allow itself to be forgotten, and in the same letter, June 15th, he writes: "This corner has we are told a

bad reputation. In front of our dug-out is a tablet of chalk smoothed and polished, and surrounded by an arch, formed by cutting away and smoothing off the rough clay side of the trench, but on the chalk is cut in relief a cross and scroll, and inscribed in pencil the name of a corporal of the —th Batt. Lon. Regt., 'Killed in this spot, June 10th.' A find which I made yesterday has made me wonder whether I have had a narrow escape, or whether a comrade has been employing his spare time in playing a practical joke. On coming into my dug-out after a short absence yesterday afternoon, I found lying on the floor just where I sit a German bullet, which had undoubtedly been fired through a German rifle, as evidenced by the rifling marks; some inches above it in the wall of the dug-out was a small hole, evidently made by the bullet, the hole being just at my elbow as I write."

In October, 1915, he writes home: "An old couplet has been much in my mind lately; perhaps because of its very inappropriateness. It is the couplet on that candlestick of mine: 'Be the day weary or be the day long, at last it bringeth to evensong.' It is the bringing to evensong that we most dislike. It means the straining of eyes and ears on sentry-go. Yes! And the straining, too, of nerves, when even the posts of our own barbed wire become approaching enemies, when a head of milfoil blossom waving in the wind tries to lead us to imagine it the grey cape of the Germans."

And through it all he reads and discusses "Les Miserables," the "Happy Warrior" and "The Siege of Paris," and never for long forgets his friends the plants. "Is it not the limit that after cherishing a plant (*Mamillaria elongata*) for seven long years, it should go and bloom just when I am away from home? How I am longing to get back to the plants and the diseases."

A spirit like this coupled with an unusual combination of practical and scientific knowledge augured well for the future spent as he hoped in combating plant disease. By the death of John Hart the country lost a valuable soldier equipped alike for Peace or War.

E. N. T.

¹ The extracts are from letters in the possession of the writer or from those kindly lent by his mother.

REVIEW.]

CAMBRIDGE BOTANICAL HANDBOOKS (Edited by A. C. Seward and A. G. Tansley). Vol. I. ALGÆ (*Myxophyceæ*, *Peridineæ*, *Bacillarieæ*, *Chlorophyceæ*), by G. S. WEST. CAMBRIDGE UNIVERSITY PRESS, 1916. (Pp. VIII and 475, with 271 figures in the text). PRICE 25s.

THE decision of the Cambridge University Press to issue a series of handbooks, dealing in a comprehensive manner with the biology of the different groups of the plant kingdom, will have been welcomed by all interested in the progress of botanical science. There are sufficient able experts in the United Kingdom to make such a series one of considerable value, and one which should have a world-wide circulation. The first of these handbooks to appear is Professor West's volume on Algæ, and we have nothing but praise for the style of production. It is, however, extremely unfortunate that the cost of the volume—and presumably of those which are to follow—is so high as to preclude a really extensive sale. Even at the sacrifice of some of the munificence in production, a considerably lower price should have been possible, ensuring, as it undoubtedly would, a much wider circulation.

Professor West approaches his subject with an authority based on many years' experience, and no one probably is so well suited as he to write on the difficult group of the Algæ. The present volume only deals with part of the group, the remaining subdivisions forming the subject-matter of a second volume. Every algologist is cognisant of the great difficulties that lie in the way of a collective treatment of this varied and in part imperfectly known group, and one may cordially congratulate Professor West on having, on the whole, encompassed the task successfully. The scope is wide, the presentation lucid, and the numerous illustrations (of which more than one-half are by the author himself) excellent and well chosen. The most serious criticism to be made is that there is frequently too much detail of a systematic nature and often a lack of sufficiently wide morphological treatment. The volume will form a first-class introduction to taxonomic work, but the comparative morphologist will occasionally find himself somewhat disappointed. It is to be regretted that Professor West decided against an inclusion of Flagellata in these volumes. Recent work has brought to light so marked a degree of parallelism between certain series of Algæ and the different subdivisions of the Flagellata, that an account of the latter is almost necessary to display clearly the general trend of Protistan evolution in the direction of the plant kingdom. We think that justice could have been done to the essential features of Flagellate morphology without very unduly enlarging the size of the work.

The volume opens with an admirable account of the Myxophyceæ (*Cyanophyceæ*), really the first modern description of the group; a particularly good section is that dealing with the distribution and habit of these Algae (pp. 30-38). With the author's conclusions as to the nature of heterocysts (p. 20) and the position of the group as a whole (p. 39) we are in complete agreement.

Griffith's work is regarded as definitely establishing *Glaucocystis* as a member of the Blue-green Algæ (p. 452), but until its cytological characters have been compared with those of the species of *Oocystis*, we do not consider that a final conclusion is possible. The statement on p. 3, as to the blue colouration of the sheath with chlor-zinc-iodide in *Schizothrix*, is misleading, as this character is not restricted to the genus mentioned, being encountered also in *Lyngbya*, *Phormidium*, etc. It is also our experience that the heterocysts are quite commonly deeply pigmented in tropical Myxophyceæ (cf. p. 17).

In the able account of the Peridineæ which follows, the author adopts Kofoid's terminology for the plates of the Peridiniaceæ. The omission of Murray and Whitting's paper from the bibliography is curious, and we are also astonished to find no reference to Pascher's (cf. Ber. Deutsch. Bot. Ges., XXXII., 1914, p. 151) description of a filamentous member of the Peridineæ (*Dinotrix*) with swimmers of the *Gymnodinium*-type. Although Pascher's communication is only a brief, preliminary one, and may rest on a misconception, it is surely important enough to deserve some attention.

The description of the Bacillariales is again quite excellent and the choice of illustrations admirable. A brief section dealing with methods of culture will prove very useful, and a similar one might well have been incorporated in the accounts of the previous groups. In discussing the affinities of Diatoms (pp. 119, 120) Pascher's views (*loc. cit.*, pp. 146, 147) on relationships with Heterokontæ and Chrysophyceæ might well have found mention.

It is to be regretted that the author adopts a different treatment, namely that of partition into numerous short sections, in the case of the Chlorophyceæ, since the many points of contact between the different subdivisions are thereby much obscured, so that it is difficult to obtain a clear view of the group as a whole. Except in the excellent account of the Conjugatæ, this is often only too apparent. Many will be inclined to disagree with the author's inclusion of the Heterokontæ as one of the four subdivisions of the Chlorophyceæ. All the evidence points to the Heterokontæ being a phylogenetic line quite separate from that of the other green Algæ. We also regard the adoption of the terms Akontæ and Stephanokontæ as undesirable, since there is nothing to show that the ancestry of these groups is really distinct from that of the Isokontæ, of which they appear to be early offshoots. The absence of motile reproductive cells in the Conjugatæ is paralleled in other series of the Isokontæ, and the large size of the Oedogoniaceous zoospore is probably responsible for its numerous cilia.

In the subdivision of the Isokontæ all the unicellular and colonial types are included in one order, the Protococcales. The author's disinclination to recognise the motile forms as a separate series, the Volvocales (as advocated by Pascher and others), is not altogether to be reconciled with the adoption of Ulvales and Schizogoniales as distinct subdivisions. The former appear merely as a specialised branch of the Ulotrichales. The axile chloroplasts and other peculiarities of the Schizogoniales do not seem to warrant separation from the Ulotrichales, since plastids of this type are

not unknown in the Protococcales (e.g. in *Asterococcus*) and are not there regarded as of marked taxonomic value.

Brief attention may be given to certain other points in the arrangement of the Isokontæ. *Sphærella* and *Stephanosphæra* are separated from the Volvoeaceæ, as the Sphærellaceæ, a change which appears quite warranted. The subdivision of the palmelloid Protococcales into Chlorangiæ, Palmelleæ, Tetrasporeæ, Palmophylleæ, and Gloeocysteæ brings out clearly the affinities of the different forms, but we should have preferred to include also the Dictyosphæriaceæ which the author regards as a separate family. We doubt the advisability of placing *Protococcus* (the name now adopted for *Pleurococcus*) in the same family (Protococcaceæ) as *Trochiscia* and *Chlorella*, since the method of propagation in the former is mainly by vegetative division, which is not encountered in the other two genera; there also appears to be certain differences in the chloroplasts. The position of *Pleurococcus* is of course still very obscure, and this is partly owing to incomplete discrimination between the species of this genus and those of *Chlorella*, *Chlorococcum* (which the author now recognises as an independent genus, cf. p. 209), and *Chlorosphæra*, but the present writer is much inclined to adopt the view propounded by some algologists that *Pleurococcus* is a reduced member of Chætophoraceæ. In any case he is not disposed to agree that the species of *Chlorosphæra* "are the relics of intermediate forms between *Protococcus* and the lowest form, viz. *Chlorococcum*, of the Chlorochytriæ" (p. 195). *Chlorosphæra* is certainly allied to the latter, but we doubt if it is to the former. The inclusion of *Chætopeltis*, *Chætosphæridium*, etc., in the Protococcales (family Chætopeltidaceæ) may be justified, although here also we are possibly dealing with reduced Chætophoraceæ. It may be doubted whether all the genera comprised in Chætopeltidaceæ really form a natural group. The suggestion that *Endosphæra*, *Scotinosphæra*, *Chlorocystis*, *Stomatochytrium*, and *Centrosphæra* (cf. p. 454) be merged in the genus *Chlorochytrium* will probably find whole-hearted approval from most algologists.

In his treatment of the Siphonales (in which *Vaucheria* and *Dichotomosiphon* are rightly included) *Halicystis* is placed together with *Protosiphon* in the family Protosiphonaceæ. We are fully in agreement with this step, in fact we think the scope of the Protosiphonaceæ might with advantage be still further enlarged. We are glad to find that the author now includes *Protoderma* in the Chætophoraceæ and that *Rhaphidonema* is recognised as an independent genus, although it would perhaps be better referred to Chætophoraceæ than to Ulotrichaceæ.

As regards the classification of the Heterokontæ the merging of Pascher's Heterochloridales, Heterocapsales, and Heterococcales in one order is open to criticism, as also the inclusion of forms like *Chlorosaccus*, *Mischococcus*, and *Stipitococcus* in one family.

Two phylogenetic points call for special consideration. The author follows Blackman and Tansley and others in deriving the Ulotrichales from the palmelloid Tetrasporineæ (cf. pp. 158, 281). It may be that this view is justified, although there is little direct evidence, but we think that Oltmanns' suggestion as to a possible

derivation from Protococcaceous forms should have found mention. The genus *Chlorosphaera*, combining as it does occasional vegetative division (in one species even with the formation of short filaments) with the customary propagation by zoospores, could equally well have been the starting-point of a filamentous series.

The author upholds his earlier view that the Desmids arose from a filamentous ancestry, although it cannot be said that the evidence brought forward is at all conclusive. The statement on p. 377 that "in *Desmidium cylindricum* and in the presumed 'abnormal' cases of conjugation in *Hyalotheca dissiliens* one is probably witnessing the type of conjugation which was prevalent in the ancestors of the Desmidiaceæ," might equally well be regarded as evidence of a specialisation accompanying the adoption of a colonial habit. Lower down on the same page the author himself describes the filamentous condition as secondary. We are more inclined to regard Desmidiaceæ and Zygnemaceæ as divergent lines from a common ancestry, and in this connection Oltmanns' distinction of the family Mesotæniaceæ (which should certainly include *Netrium*, and possibly also *Gonatozygon* and *Genicularia*¹) was in our opinion a great advance. This family, including as it does forms with all the main types of chloroplast-structure found in Zygnemaceæ, might well be regarded as a remnant of the ancestry of the Conjugatæ. The behaviour of the zygospore on germination, as far as it is known, and the simple character of the cell-wall would be quite in accordance with this theory.

There are a number of minor phylogenetic points that call for mention. We do not understand why *Gonium* is placed on a side-branch, whilst *Pandorina*, *Eudorina*, etc., are put in line in the phylogenetic scheme on p. 182. Seeing that all Volvoceæ pass through a *Gonium*-stage in the development of their daughter-colonies (a fact which is not mentioned), it would seem that *Gonium* should naturally come in line with the others. Nor is it clear why, on p. 282, *Aphanochæte* is placed as an offshoot from the line of evolution of *Draparnaldia*; to our mind the former genus is best derived from forms like *Stigeoclonium* (a name now readopted in place of *Myxonema*) by suppression of the upright branches and specialisation in the reproductive process. *Stichococcus* is probably better regarded as a reduced member of Ulotrichaceæ than as being on the upgrade of evolution, and the position of *Geminella* in the phylogenetic scheme may also be questioned. We are not prepared to accept the statement that "the principal members of the Chætophoraceæ have doubtless originated directly from Ulotrichaceæ by the branching of the thallus" (p. 293); it seems more likely that they diverged from the line of evolution of Ulotrichaceæ at a very early stage.

In the treatment of the Chlorococcineæ some mention might have been made of points of contact with Fungi like *Rhodochytrium* and *Synchytrium*. Similarly, in dealing with *Vaucheria*, some reference to the views that have been held as to its possible relationship with the ancestry of Oomycetes might have been incorporated.

¹ Making it synonymous with West's Saccodermæ.

As regards the morphological features of Chlorophyceæ, apart from the main criticism already made, we have not much to add. We are astonished to find no reference to Senn's work on Protococcales, nor to Berthold's "Verzweigung einiger Suesswasser-algen." The revelant papers are also not included in the bibliography; other important contributions that we think should have been cited are Heinricher's on *Sphæroplea* and Juranyi's on *Oedogonium*. In dealing with the Chlamydomonadeæ some reference should have been made to the relatively permanent *Palmella*-stages of certain species of *Chlamydomonas*. The statement on p. 304 that *Microthamnion* is not attached at any time is surprising, as we have certainly found it growing as an epiphyte on other filamentous Algæ.

We have already commented on the very clear presentation of facts by the author, and we have noticed but very few failings in this respect in the section on Chlorophyceæ. Attention may be drawn however to the confusing reference to fig. 149 on p. 127, to the remarks on the same page about differentiation of vegetative cells, where a mention of *Draparnaldia* would have seemed advisable, and to the statement on p. 301 as to incrustation with lime of the thallus in *Pleurothamnion*, which is not only the genus of the particular sub-family exhibiting this phenomenon.

Professor West concludes the volume with a section on the occurrence and distribution of freshwater Algæ, and a number of data of a similar kind are included in various other portions of the work. The author's classification of the Algæ according to habitat will be of great value to the algal student, but we cannot help feeling that with Professor West's wide experience a more truly ecological account of freshwater Algæ would have been possible. In particular we miss any considerable attempt at analysis of the factors appertaining to the different habitats; some reference should also have been made to Comère's work in this direction. It is stated on p. 427 that according to Fritsch and Rich the autumnal phase of *Spirogyra* is the result of the germination of a certain percentage of the zygospores formed in spring. It has however escaped Professor West's notice that the authors in question in their subsequent papers have corrected this statement and now regard the autumnal phase as solely due to persistence of a number of vegetative filaments.

F.E.F.



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THE EFFECT OF WEEDS UPON CEREAL CROPS.

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ONE of the great problems with which a farmer has to deal is the presence among his crops of weeds or plants other than those sown, which are inimical to the obtaining of maximum results. It is impossible to sow any crop without the certainty that other plants will appear, derived either from seeds already buried in the soil or from alien seeds introduced with the crop. The practical results of the competition of weed and crop are well known, but the exact cause of this competition is less obvious. The weeds utilise food and water from the soil, and above ground they tend to shade the crop and rob it of much of the sunlight essential to full development. The question constantly arises, however, as to whether this mere "vegetative competition," if it may be so termed, is the only factor at work, or whether the weeds excrete from their roots some poisonous substance which actively inhibits the growth of the crops. Fletcher^{1 2} has carried out various experiments in India and Egypt and maintains that some species are actively injurious to others owing to toxic excretions from the roots. The fruit farm experiments at Woburn indicate that growing grass round fruit trees is harmful to the growth of the trees, but Dr. E. J. Russell,³ in discussing various experiments on the subject, indicates that if a toxic substance is excreted, it is extremely unstable, and "changes into something which is harmless, or may even be beneficial to plants," and that "certain experiments made at Rothamsted by

¹ Fletcher, F., 1908. *Memoirs of the Department of Agriculture in India*. Vol. II, No. 3 (Bot. Ser.).

² *Ibid*, 1912. Toxic Excreta of Plants. *Journ. Agric. Science*, Vol. IV, pp. 245—247.

³ Russell, E. J. Effect of one Growing Crop on Another. Report XIV, Woburn Experimental Fruit Farm, pp. 63, 66.

Dr. Hutchinson indicate the possibility that bacterial activity may play a part in determining the injurious effects of the grass roots, for apparently, when the experiment was carried out under rigidly aseptic conditions, no harmful effects were obtained."

Altogether, the whole matter is very undecided, and it is as yet impossible to formulate any definite statement on the matter.

For the past four years pot experiments have been carried out at Rothamsted with crops and weeds growing in association, wheat, barley and buckwheat being used as crop plants, *Alopecurus agrestis*, *Brassica alba*, *Papaver Rhæas*, *Spergula arvensis* being selected as weeds on account of their great prevalence on certain types of soil. All the experiments were carried out in the same pots under similar open air conditions.

EXPERIMENT 1, 1913.

Soil was taken from a wide path in Little Hoos field, which had not received any special treatment or manuring. The soil was sifted to remove large stones, and half of it (A) was mixed with 10% of coarse sand to render it more workable, the other half (B) receiving 35% of sand to make the texture appreciably lighter. Thirty pots were filled with each lot of soil so prepared. Large glazed earthenware pots were used, weighing about 70 lbs. when filled, and seeds of wheat and various weeds were sown on March 31st in sets of six pots each. The pots with wheat or weed alone received twice as much seed as was sown in the mixed pots; *i.e.*, two parts of wheat or weed when alone, and one part wheat + one part weed when mixed. The following combinations were grown:—

A. soil. *Papaver Rhæas* + *Papaver Rhæas*, *Papaver Rhæas* + Wheat, Wheat + Wheat, Wheat + *Alopecurus agrestis*, *Alopecurus agrestis* + *Alopecurus agrestis*.

B. soil. *Brassica alba* + *Brassica alba*, *Brassica alba* + Wheat, Wheat + Wheat, Wheat + *Spergula arvensis*, *Spergula arvensis* + *Spergula arvensis*.

The Wheat used was the same as that sown on Broadbalk the previous autumn—Squarehead's master. The *Alopecurus agrestis* was seed procured from Broadbalk in 1912, *Brassica alba* was gathered from a field on Westfield Common, Harpenden, *Papaver Rhæas* was obtained partly from Rothamsted fields and partly from Sutton, *Spergula arvensis* was from Sutton. The *Papaver Rhæas* from Sutton was not altogether true to type—one pot sown with it yielded plants of a garden form, and so had to be rejected in recording the results.

The seeds were planted in eight clumps round each pot, the wheat and weed being placed alternately in the mixed pots. Two wheat seeds were dibbled in each hole, the weaker plants being thinned out later, and a small pinch of weed seed was likewise dibbled in per hole, but no thinning was done, all the plants being allowed to take their chance of reaching maturity. The pots were placed in the open air under the shelter of a gauze cage to protect them from the depredations of birds. Water was supplied when necessary, and the crops were harvested on August 5th and 6th. The shoots were cut off as close to the surface of the soil as possible. Then, pot by pot, the earth was carefully thrown out on to a rick-cloth and was sifted to disengage the roots as far as possible. The soil was then returned to the original pots in readiness for the second crop (Experiment 2). Prior to harvesting, the heights of the various plants were taken and the number of fruiting heads or ears recorded for each pot. The time of cutting was determined by the fact that the weeds had all reached maturity and were ripening their seeds, though the wheat was still immature and had not been long in ear.

SOIL A. TOTAL DRY WEIGHTS OF CROPS IN SIX POTS.

Shoot.			Root.
Crop.	Weed.		Wheat + Weed.
Grams.		Grams.	Grams.
	Papaver + Papaver	52.4	11.0
82.2	Wheat + Papaver	11.9	(5 pot only) 26.0
89.4	Wheat + Wheat		32.7
70.8	Wheat + Alopecurus	22.2	29.3
	Alopecurus + Alopecurus	75.0	15.7

AVERAGE DRY WEIGHTS OF SHOOTS.

Crop.		Weed.	
Per plant.	Per single sowing.	Per single sowing.	
Grams.	Grams.		Grams.
		Papaver + Papaver	5.24
3.425	13.7	Wheat + Papaver	1.98
1.863	7.45	Wheat + Wheat	
2.950	11.8	Wheat + Alopecurus	3.70
		Alopecurus + Alopecurus	6.25

(A single sowing is the quantity of wheat or weed sown in the mixed pots).

Wheat and Papaver Rhæas.

The **poppy** laboured under the disadvantage of growing on a heavy soil instead of on the light sandy medium with which it is usually associated. Nevertheless, when grown alone, good fruiting plants with well-developed roots were produced, the average height of the clumps per pot ranging from 32—38 cms., the total number of heads per pot from 30—51 (average 46). When grown with wheat very poor development was made, and in many cases the clumps failed to grow at all. The fruiting heads were very small and poor, only 3—7 (average 5) being formed per pot, and the average height of the clumps was rather low, ranging from 23—31 cms. In one pot all the poppies failed except three tiny plants of 3 cms. high. The roots in all cases were very poor, and so small that sometimes they were not found when the soil was overhauled. The dry weights clearly show the depreciation of growth in the presence of wheat.

Wheat developed better with poppy than by itself, the average weight of the plants being nearly double in the first case; the roots were good and fibrous in all the plants. When grown alone the average height per pot was 48—55 cms., the total number of ears in the six pots being 45, of which only 23 were fully developed and free from the sheath. When mixed with poppy the average height per pot was greater, 56—65 cms., and by half the number of plants practically the same number of ears were formed—42 altogether, of which 36 were fully developed. The dry weights bear out the observations made on the growing plants, as the total weight of wheat in the mixed pots is not very much less than that of twice the number of plants grown alone.

Wheat and Alopecurus agrestis.

Alopecurus agrestis or Black Bent is a denizen of heavy soils, so that it was not handicapped in operations against the wheat by a disadvantageous substratum, as was the poppy. Nevertheless, it was not able to hold its own. When grown alone the roots were good and well-developed, and each pot produced 57—78 fruiting spikes, 391 altogether—an average of 8 per clump. When grown with wheat the roots were very variable, ranging from well-developed roots almost equal to good wheat roots down to very poor scrubby specimens. The height of the clumps was hardly affected, 32—43 cms. as against 35—41 cms. when grown alone. 7—28 spikes were formed per pot, total 108, an average of 4·5 spikes per clump, showing a marked

decrease on the figures when grown alone. The dry weight also dropped considerably. It is interesting to note that the detailed analysis of weight, number of spikes and root development reveals a far greater depreciation in the growth of *Alopecurus* with wheat than was evident during the growing period. Although some depression was evident, it looked comparatively slight, and the analytical figures, revealing a depression of some 40% in the presence of wheat came in the nature of a surprise.

The **wheat** was as deceptive as the *Alopecurus*. It appeared to be very poor and backward (except in one pot) and to be most adversely affected by the weed. As a matter of fact the height of the plants was rather low, 26—39 cms. (reaching to 62 cms., in the good pot) and this probably gave rise to the impression of poor development. The roots were quite well-developed and fibrous. Altogether 30 ears were formed, of which 21 were fully developed, as against 45 and 23 from twice as many plants grown alone. In proportion the dry weights of these wheat plants were far heavier than in the absence of the *Alopecurus*, though the totals did not approximate quite so closely as with wheat and *Papaver*.

SOIL B. TOTAL DRY WEIGHTS OF CROP IN SIX POTS.

Shoot.		Root.
Crop.	Weed.	Wheat + Weed.
Grams.	Grams.	Grams.
36.6	Brassica + Brassica	7.0
70.7	Wheat + Brassica	10.6
57.9	Wheat + Wheat	27.4
	Wheat + Spergula	21.4
	Spergula + Spergula	1.9

AVERAGE DRY WEIGHTS OF SHOOTS.

Crop.		Weed.
Per plant.	Per single sowing.	Per single sowing.
Grams.	Grams.	Grams.
1.525	6.1	Brassica + Brassica 4.27
1.473	5.89	Wheat + Brassica 4.22
2.413	9.65	Wheat + Wheat
		Wheat + Spergula 0.32
		Spergula + Spergula 3.3

Wheat and Brassica alba.

With this association matters were most evenly balanced, as the analyses give very similar results whether the plants were grown alone or in conjunction. The heights of the **wheat** and *Brassica*, the number of wheat ears formed and fully developed the average dry weights of the shoots of both plants were all practically the same in every case. The only indication of unequal competition was shown by the roots. *Brassica* roots alone were only fairly well developed, whereas wheat roots alone were good and fibrous. When associated the *Brassica* roots were medium, while the wheat roots were remarkably poor, with a great lack of fibre. It is curious that this deficiency in the root development of wheat was not reflected in the shoots; possibly some difference might have manifested itself had the plants been allowed to grow on and ripen their grain.

Wheat and Spergula arvensis.

Spergula, when grown alone, was variable but fairly strong in the shoot, though the roots were small; the average height per pot ranged from 16—28 cms. When grown with wheat it was very poor indeed, average height 7—10 cms., and the roots were not to be seen in the soil. The fall in dry weight was very great, being from 100% to 10% for equal numbers of clumps.

The **wheat** was more influenced by the presence of the spurrey than would have been expected from the small amount of growth made by the weed. The roots were somewhat variable (mostly good, some medium) instead of being universally good and fibrous, and, although the average dry weight of the plants was considerably heavier, yet the number of ears produced was the same for equal numbers of plants, 18 being formed, of which 11 were fully developed. It must be remembered that the wheat was somewhat handicapped, as soil B contained so much sand that it was of a distinctly light texture, which is detrimental to the best development of the wheat plant.

Summing up these results it seems that definite interactions take place when wheat, poppy, *Alopecurus*, *Brassica* and spurrey are grown alone or when one weed is associated with wheat. All five made satisfactory growth by themselves. When wheat was grown with poppy, *Alopecurus* or spurrey the weed suffered in various ways, particularly in the reduction of dry matter and in the

capacity for producing fruiting heads or spikes. The wheat, on the contrary, gained by the association, being 60—85% heavier, plant for plant. This shows that the competition is not confined to that between wheat and weed, but that it also exists between the wheat plants themselves. When the weeds are so badly handicapped that they make little growth, the wheat plants have a comparatively clear field and are able to make more growth than when the place of the weed is taken by another wheat plant.

As the spurrey made so very little growth when with wheat it might have been expected that the average dry weight of the wheat would have increased more than the 65% that it actually did, seeing that with poppy, on soil A, the increase reached 85% in the presence of a greater amount of weed. This may possibly be accounted for in two ways:—

(1) Soil A is far more favourable to the growth of wheat, so that probably greater increase in development was possible in it.

(2) The spurrey grew fairly well at first, and its habit is to spread and cover the surface of the ground, unlike poppies which grow erect. It may be that in the early stages of growth the spurry offered a real competition to the young wheat plants, checking them to a certain degree by robbing them of some amount of light. If such a preliminary check did take place, it is conceivable that its influence might be felt all through the life of the plant, so that the latter could never develop quite so well as if it had grown unhindered from the start.

Brassica and wheat, under the conditions of this experiment, seemed to be on equal terms. It is evident that competition exists between the *Brassica* plants as between the wheat plants, and in much the same degree, so that when the two were grown together the basis of competition was simply shifted without any further detriment to the growth of the individual plants.

From this first experiment there is no evidence that any factor is in play other than that of competition, as all the phenomena can be explained by the fact of competition without the necessity for presupposing any more active factor such as that of toxic root excretions.

EXPERIMENT 2, 1913.

As before described (Experiment 1) the soil was returned to the original pots when the roots had been removed, and the pots so prepared were utilised for a second crop in the same year. Buck-

wheat and the same weeds as before were sown on August 8th; again eight clumps were put in per pot, two buckwheat plants being allowed to come up together; spurrey and *Brassica* were dibbled in as before, but the poppy and *Alopecurus* were scattered in little patches where the clumps were to be established. The plants were harvested on October 23rd, the roots again being removed from the soil. It was noticed that on the lighter soil the buckwheat looked better when grown in association with weed than when alone, but on the heavier soils the plants were comparable in appearance whether they were grown mixed or alone. It will be seen that these observations were borne out by the dry weights.

SOIL A. TOTAL DRY WEIGHTS OF CROP IN SIX POTS.

Shoot.			Root.
Crop.	Weed.		Crop+Weed.
Grams.		Grams.	Grams.
	Papaver+Papaver	5.4	0.6
25.0 Buckwheat+Papaver		0.31	1.2
35.9 Buckwheat+Buckwheat			1.6
27.4 Buckwheat+Alopecurus		0.58	1.5
	Alopecurus+Alopecurus	5.1	1.25

AVERAGE DRY WEIGHTS OF SHOOTS.

Crop.		Weed.	
Per clump.	Per single sowing.	Per single sowing.	
Grams.	Grams.		Grams.
		Papaver+Papaver	0.45
1.042	4.167	Buckwheat+Papaver	0.052
0.748	2.992	Buckwheat+Buckwheat	
1.142	4.567	Buckwheat+Alopecurus	0.097
		Alopecurus+Alopecurus	0.425

Buckwheat and Papaver; Buckwheat and Alopecurus.

The growth of the weeds, even when alone, was very irregular and poor, probably on account of the lateness of sowing. The **poppy** plants were somewhat mixed, the *Papaver Rhæas* only reaching a height of about two inches, the other species attaining six inches. **Buckwheat** made quite good growth.

The Effect of Weeds upon Cereal Crops. 61

In both cases the presence of buckwheat almost killed out the weed, both poppy and *Alopecurus* being very poor. The **buckwheat** itself profited by the increased space available, and increased by some 40%—50% in dry weight. It seems as though in this case the increase must be due to the removal of aerial competition, as the buckwheat roots are so fine and the pots were so large that it hardly seems possible that competition between the roots can have come seriously into play.

SOIL B. TOTAL DRY WEIGHTS OF CROP IN SIX POTS.

Shoot.		Root.
Crop.	Weed.	Crop + Weed.
Grams.	Grams.	Grams.
27.0	Brassica + Brassica	20.6
20.4	Buckwheat + Brassica	4.5
29.2	Buckwheat + Buckwheat	2.5
	Buckwheat + Spurrey	3.9
	Spurrey + Spurrey	

AVERAGE DRY WEIGHTS OF SHOOTS.

Crop.		Weed.
Per clump.	Per single sowing.	Per single sowing.
Grams.	Grams.	Grams.
1.152	4.5	Brassica + Brassica
0.284	1.7	Buckwheat + Brassica
1.215	4.8	Buckwheat + Buckwheat
		Buckwheat + Spargula
		Spargula + Spargula

Buckwheat and Brassica alba; Buckwheat and Spargula.

In this lighter soil the **buckwheat** grown by itself shows a remarkable drop in the dry weight when compared with that in the heavier soil, from 100% to about 57%, although when grown with the weeds it reaches an almost equal development in both soils. As a result the actual weight of the eight clumps grown alone is considerably less than that of the four clumps grown in association with the weeds. No explanation of this can be put forward.

Buckwheat and Brassica.

The *Brassica* was the only weed that made thoroughly good growth at this late date; it is one of those plants that apparently

germinates satisfactorily at any time of the year, so that the season of the experiment made no difference to it. By itself good progress was made; with buckwheat very great deterioration was observed.

Buckwheat and Spargula.

During the progress of the experiment the **spurrey** looked quite as good when associated with buckwheat as when grown alone, and this was corroborated by the dry weights—the advantage being on the side of the associated plants. This is the only case so far met with in which an associated weed has made better growth than when grown alone, but too much weight must not be placed on this instance, as the growth of the spurrey was not good, so that comparison is not altogether fair or accurate. It does suggest, however, that under certain circumstances the association of two species may be to the advantage of both—that for some reason it may happen that the competition between diverse species is less keen than that between plants of one and the same species.

EXPERIMENT 3, 1914.

Fresh soil was used in this experiment, thirty pots being filled with heavy soil from Little Hoos field, mixed with a little sand to make it more workable, the other thirty pots being filled with light sandy soil specially obtained from Woburn Experimental Station, through the kindness of Dr. J. A. Voelcker. Seeds were sown on April 17th, wheat being used as the crop plant. *Papaver* and *Spargula* were grown in the Woburn soil and *Brassica* and *Alopecurus* in the Rothamsted soil. The same treatment was given to the pots as in the previous experiments, any alien weeds arising from seeds buried in the soil being taken out as soon as observed. At the time of harvesting it was impossible to turn out the soil in order to extricate the roots, so the crops were cut off close to the surface and the roots were perforce neglected. The difference in the growth of the wheat on the two soils was very marked; that on the Rothamsted soil had a greater average length of straw and ear, and in addition produced a greater number of ears, 55 as against 31 on the Woburn soil. The greatest difference, however, was in the dry weight, the Rothamsted soil plants grown alone weighing 125 grams against the 99 grams of the Woburn soil plants. This result was only to be expected, as the heavier soil naturally produces a better crop of wheat under ordinary field conditions.

WOBURN SOIL.

TOTAL DRY WEIGHTS OF CROPS FROM SIX POTS.			
Crop.		Weed.	
Grams.			Grams.
73.3	Wheat	Papaver + Papaver	74.3
99.4	Wheat + Wheat	Papaver	10.1
76.7	Wheat	Spergula	32.4
		Spergula + Spergula	106.4

AVERAGE DRY WEIGHTS OF SHOOTS.			
CROP.		WEED.	
Per plant.	Per single sowing.	Per single sowing.	
Grams.	Grams.		Grams.
3.055	12.22	Wheat + Papaver	6.19
2.07	8.28	Wheat + Wheat	1.68
3.195	12.78	Wheat + Spergula	5.4
		Spergula + Spergula	8.87

Wheat and Papaver.

The results were similar to those obtained in the previous experiments. The **poppy** grown with wheat was far shorter and less developed than that grown alone, reaching a height of 14—23 cms. against 33—58 cms., this poorer development being associated with a heavy drop in dry weight. The **wheat**, again as before, was much stronger when in conjunction with the poppy, the average dry weight of the plants being much heavier than when grown alone.

Wheat and Spergula.

Spurrey was growing on a soil specially well adapted to it, as it is one of the natural weeds on the light sandy soil at Woburn. Consequently the plants grown by themselves made good growth, reaching a height of 33—42 cms., and making much weight. In conjunction with wheat the plants were shorter, 16—40 cms., but although the development was not so good as in the absence of wheat, still the drop in dry weight was far less marked than in most of the experiments. In spite of this relatively good growth of the weed, the **wheat** grown with it again made a very heavy average weight when compared with that grown alone.

LITTLE HOOS SOIL.

TOTAL DRY WEIGHTS OF CROPS FROM SIX POTS.			
Crop.		Weed.	
Grams.			Grams.
		Alopecurus+Alopecurus	90.6
126.0	Wheat+Alopecurus		13.0
125.6	Wheat+Wheat		
74.9	Wheat+Brassica		30.5
	Brassica+Brassica		49.1

AVERAGE DRY WEIGHTS OF SHOOTS.			
Crop.		Weed.	
Per plant.	Per single sowing.	Per single sowing.	
Grams.	Grams.		Grams.
		Alopecurus+Alopecurus	7.55
5.2	21.0	Wheat+Alopecurus	2.17
2.618	10.47	Wheat+Wheat	
3.12	12.48	Wheat+Brassica	5.08
		Brassica+Brassica	4.091

Wheat and Alopecurus.

The results were exactly similar to those obtained in the earlier experiments. When the two plants were associated there was a very heavy drop in the weed crop, while the wheat practically doubled its weight. The height of the *Alopecurus* was less and that of the **wheat** was much the same when associated than when alone.

Wheat and Brassica.

The results recalled those of Experiment 1. As a matter of fact, both wheat and weed did rather better when they were associated than when they were subjected to the competition of their own species, the average dry weights of both wheat and brassica being somewhat higher in the mixed pots than in those with pure cultures. It is evident that in soil which is favourable to both these plants the competition between them is very even, that given equal numbers of plants the growth will be fairly equal however the species are combined. The **wheat** is not so well able to overpower the *Brassica* and reduce its growth as it is in the case of the other weeds tested—poppy, spurrey and *Alopecurus*. Consequently, if

Brassica is present in a wheat crop even in a moderate amount, it is capable of doing a great deal of damage, as the vigorous growth and the broad coarse leaves render it a much more formidable competitor, plant for plant, than many of the other weeds.

EXPERIMENT 4, 1915.

The Woburn soil used in the previous experiment was mixed with a fresh load, also brought from Woburn, and 48 pots were filled with the mixture. A corresponding set of 48 pots were filled with heavy Rothamsted soil from Stackyard field, mixed with 10% of sand, to make it more workable. In the previous tests the seeding of the pots had been similar and equal all through the series. This time it was decided to try the effect of both light and heavy seeding in order to ascertain whether the competition between plants of the same species is as severe as that between plants of different species. To this end the following scheme of seeding was adopted, six similar pots being the unit in each case.

Wheat + Wheat.

Wheat.

Wheat + Weed.

Weed.

Weed + Weed.

Seeds were sown on April 20th. The wheat seeds were counted and were dibbled in at regular intervals round the pots. The smaller weed seeds were weighed out, the larger were counted, and all were scattered broadcast in order to give the roots of the weeds a better chance of coming into direct contact with the wheat roots. The following quantities were sown per pot :—

Single sowing.

Wheat 8 seeds, thinned to 4.

Papaver 0·015 gram.

Spergula 0·045 gram.

Alopecurus 20 seeds.

Brassica 20 seeds.

Double sowing.

Wheat 16 seeds, thinned to 8.

Papaver 0·030 gram.

Spergula 0·090 grm.

Alopecurus 40 seeds.

Brassica 40 seeds.

As before *Spergula* and *Papaver* were grown on Woburn soil, *Brassica* and *Alopecurus* on Rothamsted soil.

A number of alien weeds came up from buried seeds, but these were frequently pulled up in order to leave a clear field for the experimental plants. A few big poppies appeared from buried seeds in the Woburn soil, but as their larger size easily distinguished these from those sown, they were pulled up as early as possible.

At harvest time, July 30th, labour was short on account of the war, so again it was not possible to have the roots extricated from the soil. For the sake of accuracy the plants were pulled up (not merely cut), the roots were then cut off and returned to the pots, the shoots being dried and weighed. The wheat was far from mature. No ears had yet been produced, as the crop had been sown too late to allow for full development. For this reason the balance of opportunity was on the side of the weeds, so that any action of weed on wheat was considerably exaggerated in comparison with what actually takes place in the field; for the elucidation of the problem under consideration this is a distinct advantage.

WOBURN SOIL.

TOTAL DRY WEIGHTS OF CROPS FROM SIX POTS.			
Crop.		Weed.	
Grams.			Grams.
		Papaver	46.7 (5 pots)
		Papaver+Papaver	54.9 (5 pots)
25.8	Wheat+Papaver	Papaver	16.9
41.1	Wheat		
40.1	Wheat+Wheat		
10.0	Wheat	Spargula	62.2
		Spargula+Spargula	86.9
		Spargula	70.5

AVERAGE DRY WEIGHTS OF SHOOTS.			
Crop.		Weed.	
Per plant.	Per single sowing.	Per single sowing.	
Grams.	Grams.		Grams.
		Papaver	9.34
		Papaver+Papaver	5.49
1.08	4.3	Papaver	2.82
1.71	6.85		
0.835	3.34	Wheat	
0.42	1.67	Wheat	
		Wheat	
		Spargula	10.37
		Spargula+Spargula	7.24
		Spargula	11.75

Wheat and Papaver.

For the first month the **wheat** made good progress, and looked much the same in all cases, whether with single or double

sowing or with *Papaver*; apparently competition had not yet come into play. By the end of the second month the thickly sown plants were not so large as the others, but those with the poppy still looked full size. By harvest time, however, the latter plants seemed to have fallen very much behind the single sown plants, and were very poor indeed. The double sown wheat appeared on the whole very much like the single sown, but close inspection showed that while the total development was similar, the individual plants were much smaller. The dry weights absolutely bore out this latter observation, but curiously enough the average weights of the plants grown with poppy was somewhat higher than that of the double sown wheat, although the latter seemed to be so much poorer at the time of cutting.

For some long time the development of the **poppy** was much the same in all the pots, but by July a difference could be seen. The plants with wheat were rather smaller than those grown alone, while the total development was comparable in both single and double sown pots. The impression was certainly not given that twice as much seed had been sown in the latter case. The presence of the wheat had a harmful effect on the poppy, so that the average dry weight with wheat was considerably below that of the double sown poppy, *i.e.*, the competition of wheat with poppy is greater than that of poppy with poppy, but the competition of poppy with wheat is less vigorous than that of wheat with wheat, suggesting that in this case a certain balance of competition has been struck.

Wheat and Spargula.

Wheat associated with *Spargula* made a good start but soon began to fall behind, and by the end of the first month it looked decidedly unhappy. By harvest time the plants were very poor indeed, as they were practically smothered by the weed. In fact, the wheat made less growth in this case than in any experiment carried out along these lines.

The *Spargula* made excellent growth, that with wheat being nearly as strong as that in the single sown pots all the way through the experiment. The thickly sown crop looked very little heavier than the thinly sown, and this was corroborated by the dry weights. In this case the tables were turned, and the weed got the upper hand of the wheat, the depreciation of the latter going on *pari passu* with the improvement of the weed, so that the development of the spurrey approached within measurable distance

of that reached by the single sown plants, which were individually much better than the double sown. In this case the competition of the wheat with the spurrey was less keen than that of spurrey with itself.

ROTHAMSTED SOIL.

TOTAL DRY WEIGHTS OF CROPS FROM SIX POTS.			
Crop.		Weed.	
Grams.			Grams.
		: Alopecurus	33.4
		: Alopecurus + Alopecurus	93.8
88.7	Wheat + Alopecurus		0.1
85.5	Wheat :		
97.6	Wheat + Wheat :		
66.7	Wheat + Brassica		29.0
	: Brassica + Brassica		94.9
	: Brassica		86.1

AVERAGE DRY WEIGHTS OF SHOOTS.			
Crop.		Weed.	
Per plant.	Per single sowing.	Per single sowing.	
Grams.	Grams.		Grams.
		: Alopecurus	5.57
		: Alopecurus + Alopecurus	7.82
3.695	14.78	Wheat + Alopecurus	0.02
3.562	14.25	Wheat :	
2.032	8.13	Wheat + Wheat :	
2.78	11.12	Wheat + Brassica	4.83
		: Brassica + Brassica	7.91
		: Brassica	14.35

Wheat and Alopecurus.

The *Alopecurus* behaved in rather an abnormal way, possibly because it was sown at an unusual season of the year, so that it had rather a poor chance. When grown with wheat it practically failed—one feeble plant in one pot being the sole representative at harvest time. The best plants in every respect were in the double sown pots; these were strong and in full flower, and were far stronger individually than in the single pots. *Alopecurus* is not unusually an obnoxious weed except on certain heavy soils where for any reason it is difficult to clean the land well in autumn, or between two cereal crops. The seed ripens at the same time or

rather earlier than the wheat, falls to the ground and germinates quite early in the autumn. If another white crop follows on, so that cultivation cannot be carried out, then the *Alopecurus* has a chance of establishing itself; if rotation is followed or spring corn is sown, it is easy to get rid of the pest. These facts indicate that this weed is particular in its demands, and that it grows best with autumn sowing and with those plants that give it a certain amount of shelter in its young stages. This may account for the individual plants in the single pots being so much poorer than in the double pots—possibly the stand is too thin to provide the necessary shelter and so development fell behind.

The **wheat** behaved as usual—the plants with single sowing and with the weed that failed were individually much the same and were very much heavier than those sown more thickly, though they did not attain to quite twice the size. Under the conditions of experiment the wheat was a fatal competitor to the *Alopecurus*, though it suffered no check itself from the competition of the weed.

Wheat and Brassica.

Thinly sown *Brassica*, grown alone, made more individual growth than that sown thickly. In the presence of wheat, however, a marked fall occurred, and the individuals only made about half the growth of those in the double pots; *i.e.*, in this case, other things being equal, the competition between wheat and *Brassica* was greater than that between the *Brassica* plants themselves. The **wheat**, on the contrary, made better growth with *Brassica* than in the double sown pots, so that the effect of the *Brassica* on the wheat was less than that of the wheat itself.

EXPERIMENT 5, 1916.

The soil from each pot used in the 1915 experiment was tipped out, thoroughly mixed up and returned to the same pot. The same species and quantities of weeds were used as in the previous experiment, and they were sown in the same pots in the way previously described, so that *Papaver* followed *Papaver* and crop+*Spergula* followed crop+*Spergula*, and so on. Owing to a deficiency in supply only 11 *Alopecurus* seeds could be used for a single sowing, instead of 20, and also barley was used as the crop plant instead of wheat to get over the difficulty arising from the unavoidable lateness of sowing. As usual two barley seeds were sown per clump and thinned soon after the seeds had germinated. The

seeds were sown on April 5th, and the pots were run out into the open about May 16th, as soon as the plants were large enough and the weather conditions were favourable. The plants were harvested on July 20th, and dried and weighed as usual.

WOBURN SOIL.

TOTAL DRY WEIGHTS OF CROPS FROM SIX POTS.

Crop.		Weed.
Grams.		Grams.
	Papaver	55.95
	Papaver + Papaver	63.67
33.45	Barley + Papaver	44.66
95.72	Barley	
92.48	Barley + Barley	
33.49	Barley + Spargula	58.33
	Spargula + Spargula	116.48
	Spargula	98.50

AVERAGE DRY WEIGHTS OF SHOOTS.

Crop.		Weed.
Per plant.	Per single sowing.	Per single sowing.
Grams.	Grams.	Grams.
	Papaver	9.33
	Papaver + Papaver	5.31
1.39	Barley	7.44
3.99	Barley	
1.93	Barley + Barley	
1.40	Barley + Spargula	9.72
	Spargula + Spargula	9.71
	Spargula	16.42

Barley and Papaver.

The germination and early growth of all combinations of plants were normal and similar, but by the end of six weeks some differentiation was apparent. **Barley** in both single and double sowing was well developed, but the total growth with single sowing was almost as strong as with the double sowing even at this early date. In association with poppy the growth was far less good. As time went on these differences were accentuated, and at harvest time the double sown individuals were far less strong, possessing weaker stems, while the barley sown with poppy was very variable and poor.

The single sown *Papaver* made good heavy growth; the plants were well developed with big flower buds, and were stronger than those grown in association with barley, though the difference was not very strongly marked. With double sowing the individuals were decidedly weaker, though the total growth seemed greater than with single sowing. All these observations were fully borne out by the dry weights.

Barley and Spergula.

The *Spergula* was more harmful to the associated **barley** than was barley itself, though the barley grown with this seed was rather stronger at harvest than that grown with *Papaver*. The competition of barley or *Spergula* with *Spergula* was very equal, as the total growth per single sowing in both cases was practically identical. When free from competition *Spergula* made far more growth, being more than 65 per cent. heavier in dry weight.

ROTHAMSTED SOIL.

TOTAL DRY WEIGHT OF CROPS FROM SIX POTS.

Crop.		Weed.	
Grams.			Grams.
		Alopecurus	87.94
		Alopecurus + Alopecurus	101.57
113.24	Barley + Alopecurus		14.70
126.06	Barley		
38.86	Barley + Barley		
42.89	Barley + Brassica		62.76
	Brassica + Brassica		60.34
	Brassica		92.28

AVERAGE DRY WEIGHTS OF SHOOTS.

Crop.		Weed.	
Per plant.	Per single sowing.	Per single sowing.	
Grams.	Grams.		Grams.
		Alopecurus	14.66
		Alopecurus + Alopecurus	8.45
4.72	18.87	Barley + Alopecurus	2.45
5.25	21.01	Barley	
2.43	9.72	Barley + Barley	
1.79	7.15	Barley + Brassica	10.46
		Brassica + Brassica	5.03
		Brassica	15.38

Barley and Alopecurus.

In this case the harmful effects of the barley upon *Alopecurus* was far greater than that of *Alopecurus* upon barley. The drop in weight of **barley** due to the competition of the associated weed was less marked than with any of the other weeds used, whether in Rothamsted or Woburn soil. The *Alopecurus* was evidently far more affected by competition. It reduced its own production of dry matter by about 40 per cent., and the competition of barley caused a drop of over 80 per cent. The latter result serves to corroborate the idea suggested by the 1915 experiments that *Alopecurus* can only flourish and become a serious competitor of a cereal crop when it is able to germinate at its own special time and to get a firm undisturbed hold on the soil before the crop has had a chance of attaining any size. Consequently *Alopecurus* is not to be expected to be a serious competitor with spring crops which start right away and make strong growth within a comparatively short time.

Barley and Brassica.

The *Brassica* behaved rather differently this season. All the way through the experiment that sown with barley looked decidedly stronger than the double sown *Brassica*, though it was weaker than that sown grown alone in single sowing. With double sowing the weight dropped to $\frac{1}{3}$ that of the single sown plants, indicating a more vital competition between *Brassica* and *Brassica* than between *Brassica* and barley for this experiment.

WATER CULTURE EXPERIMENTS.

Pot experiments did not give any indication that toxic excretions from the weed roots were affecting the growth of the crops, so an initial attempt was made in 1915 to throw some light on this question by a series of water cultures, and though the immediate object was not attained, still some interesting results were forthcoming.

A fairly strong food solution was used, containing

- 1 gram. Potassium nitrate.
- 0.5 „ Calcium sulphate.
- 0.5 „ Magnesium sulphate.
- 0.5 „ Sodium chloride.
- 0.5 „ Potassium dihydrogen phosphate.
- 0.04 „ Ferric chloride.
- Distilled water to make up one litre.

The plants were grown in bottles of 600 c.c. capacity ; after three weeks extra ferric chloride was added at the rate of 0.02 grams per litre, and at the end of five weeks the food solutions were completely renewed. Two plants were grown in each bottle, the corks being bored with two holes and cut into three pieces in order to accommodate the plants. Wheat + wheat, wheat + weed, weed + weed were tested in each of two sets of experiments, the weeds being *Spergula arvensis* and *Alopecurus agrestis*. In every case ten bottles were grown with a similar combination of plants.

Wheat and Spergula arvensis.

Wheat seed, graded 0.06—0.07 gram., sown April 4th.

Spergula sown April 3rd.

Plants put into solution April 15th.

The **wheat** grew well from the beginning, either alone or with spurrey. After two or three weeks the double wheat plants were yellowish, but they soon recovered on the addition of extra iron. The plants associated with spurrey were much less etiolated at the same time. All the wheat remained strong and healthy until harvested.

The *Spergula* started into growth quite well in both cases, but within three weeks a difference manifested itself. The double plants were fine and healthy, had made strong growth, and had nearly all developed two whorls of leaves. The single plants, with wheat, were much less strong, and most had only formed one whorl of leaves. If two were present the upper whorl was weak. Apparently the overshadowing of the wheat had begun to affect growth. Before the close of the experiment on July 4th, all the spurrey plants, whether alone or associated, had died off without making much more growth. This may have been due to a temperature factor, as some very hot weather was experienced. It was impossible to disentangle the roots of the plants, so that unfortunately it was necessary to dry and weigh the roots of the two plants from each bottle together.

Great variation was observed in the weights of the different pairs of plants, the variation being much more marked than is usual in water cultures when plants are grown singly under similar conditions. The results were as follows:—

			Range of variation.	Mean.
			Grams.	Grams.
Wheat \pm Wheat	4.63—11.95	7.365 \pm 0.474
Wheat \pm Spargula	4.01— 8.65	7.037 \pm 0.340
Spargula \pm Spargula	0.44— 0.83	0.579 \pm 0.025

The weight of the spurrey plant is obviously almost negligible in the wheat and spurrey, as the latter did not develop very far, ceased growing early in the experiment, and died off before harvesting. Consequently the wheat did not suffer any very great initial check, and as soon as the competition factor of spurrey was virtually removed, the wheat took advantage of the fact, so that the individual plants of wheat with spurrey showed a gain of about 90% over the wheat which had mutual competition of wheat all along the line. It seems evident that the spurrey cannot have exercised any other appreciable influence than that of mere competition at the beginning, as if any such action had been at work to any degree the wheat could not possibly have gained so much ground.

Wheat and Alopecurus agrestis.

Wheat seed graded 0.06—0.07 gram. Sown April 7th.

Alopecurus agrestis sown April 3rd.

Plants put into solution April 18th.

The **wheat** grew well from the beginning to the end of the experiment. Some slight etiolation appeared in all plants in about three weeks, but this was soon remedied by the addition of additional ferric chloride.

Alopecurus started well, but within three weeks the plants associated with wheat appeared rather less strong than the others, and before the end of the experiment they were quite dead. The double plants, however, remained strong and healthy to the end, though they exhibited considerable variation. As with spurrey, the plants could not be separated when harvested.

			Range of variation.	Mean (per bottle)
			Grams.	Grams.
Wheat \pm Wheat	3.21—8.43	6.107 \pm 0.416
Wheat \pm Alopecurus	3.31—6.74	4.973 \pm 0.247
Alopecurus \pm Alopecurus	0.30—1.59	0.908 \pm 0.093

The weight of the *Alopecurus* grown with wheat must have been very small, so that the wheat plant itself gained considerably, though less than when spurrey was the competing plant. The *Alopecurus* developed strongly at first, so that it was probably a real competitive factor at the outset. As it died off later without making any considerable growth, the active competition was largely withdrawn, but even so the wheat failed to make any such great improvement as it did in the case of spurrey, in which the competing plant made even less growth and died off sooner. This seems to indicate either:—

(1) that the *Alopecurus* was a very real competitive factor at the beginning, so that the wheat suffered such an initial check that even when the *Alopecurus* died off it was unable to make up the lee-way so well as it did when grown with spurrey, or—

(2) that the *Alopecurus*, as well as or instead of being merely an initial competitive factor, so working its mischief, was in some way actively inimical to the wheat plant, so that when the competition was removed and the wheat had apparently full opportunity to push ahead, the deleterious factor still remained in play and prevented the development otherwise possible.

CONCLUSIONS.

All things considered, in the light of the water culture and soil culture experiments, it seems probable that the first of the above explanations is the true one. There is no evidence, and indeed no indication, that any direct toxic action comes into play. It is evident that the mere competition of plant with plant, irrespective of species, has much to do with development and that the time and duration of competitive check are the chief factors involved. Negative results are necessarily inconclusive, so that it is still impossible to say definitely whether or not such plants as *Alopecurus* are harmful to crops in any other way than that of direct "vegetative" competition. It is clear that such competition is more potent than is generally realised. In the experiments all the crop plants were at least 4 or 5 inches apart, and the effects of overcrowding were most obvious. In a field where weeds are at all prevalent, the plants are still more closely placed, and the struggle must be still keener. Even when the weeds are suppressed by cultivating and hoeing the roots remain in the soil to a large extent, and those of the perennials at least continue functioning in a normal way, though to a less degree; nevertheless, such suppression

of weeds is all to the advantage of the crop—the removal of the aerial competition enables the crop to forge ahead far more rapidly in spite of what the roots of the weeds may be doing in the soil.

If the weed roots really excreted a poisonous substance, one would have expected, when crop and weed were grown in conjunction, that the crop would have been less well developed than in the absence of weed, or, at least, that it would not have shown any increased growth. Instead of this, in nearly every case, the crop associated with the weed showed marked individual improvement, which points to the fact that the vital factor in competition is the mere presence of other plants, be they what they may, and that up to a certain limit, two plants cannot make such good individual growth in a given restricted area as can one plant.

A comparison of the dry weights, pot for pot, for the two years 1915 and 1916 does not show any definite correlation, in that it is not apparent that the crop obtained from any individual pot in 1915 in any way affected that obtained in 1916, so that the differences between the pots in each set of six must be regarded as accidental, and not as due to any inherent quality of the soil in the various pots. This is a further proof of the absence of toxic effects from the roots. If toxins had been present a pot which carried a relatively small crop in 1915 might have been expected to carry a relatively large one in 1916 and vice versa, as the large crop would have left a larger supply of toxin in the soil. As no such correlation was proved it seems evident that no toxin capable of remaining unchanged from one season to the other was present in the soils.

CARBON ASSIMILATION.

A REVIEW OF RECENT WORK ON THE PIGMENTS OF THE
GREEN LEAF AND THE PROCESSES CONNECTED WITH THEM.

By INGVAR JÖRGENSEN AND WALTER STILES.

(Continued from p. 45).

CHAPTER VII.

Theories of Carbon Assimilation.

A. GENERAL REMARKS.

It is significant to note that the contributions to the literature with which this chapter deals, are not the work of those plant physiologists who have built up this branch of their subject: de Saussure, Sachs, Pfeffer, F. F. Blackman. It seems as if those who by years of experience have obtained most insight into the complexity of plant processes have realised that the only way for development lay in bringing to light facts, and endeavouring to determine the laws underlying these facts.

It is remarkable that all the theories of carbon assimilation have not advanced the state of plant physiology in the least; it would not have materially altered our knowledge of plant processes if all that voluminous literature had never appeared. Thus none of the various aspects of carbon assimilation with which we have dealt in the preceding chapters owes anything of its development to any theory of carbon assimilation that has ever been advanced.

It is surprising that no protest has been raised by plant physiologists against the overwhelming tendency to publish theories which have little or no reference to the facts of assimilation by the plant. Spoehr's recent paper, "The Theories of Photosynthesis in the Light of Some New Facts" (1916), is indeed a voice raised in the desert. We should specially like to draw attention to this paper which critically examines one group of theories, those based on the formaldehyde hypothesis. Generally speaking, we agree with Spoehr's statement, that "It can safely be said at the outset that, when critically considered from a physiological view point, none of the existing theories is even moderately well established by observations of facts."

In the following we shall cite the theories and suggestions of various chemists who have directed their attention to the problems of carbon assimilation, namely A. Baeyer, J. H. van't Hoff, M. Siegfried, and R. Willstätter. Only the hypothesis of Baeyer seems to have aroused any interest among botanists, as the

literature of the subject sufficiently indicates, and it is not unusual to find Baeyer's hypothesis almost accepted as an axiom in biological text-books. Here again we agree with Spoehr who expresses as his opinion, "In recent years this hypothesis has largely directed the course of the investigations in this subject, and it seems to the writer, to the detriment of critical and independent thinking on the broader aspects thereof."

B. HYPOTHESIS OF BAEYER.

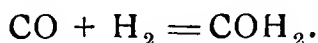
We shall in what follows only deal with Baeyer and his followers as briefly as possible, as those interested in this branch of this subject will have no difficulty in finding ample references to the literature in text-books and journals.

Before Baeyer's time the chemists, for instance, Liebig (1843b), Kolbe and Schmidt (1861), and Berthelot (1864), appear to have been of the opinion that in the process of assimilation organic acids were produced from which carbohydrates were subsequently formed. In the course of a paper, entitled "Ueber die Wasserentziehung und ihrer Bedeutung für das Pflanzenleben und die Gährung," Baeyer threw out a suggestion as to the formation of formaldehyde as an intermediate product of assimilation, a hypothesis which was really based on Butlerow's observation (1861) that trioxymethylene (a condensation product of formaldehyde) on heating in alkaline medium yields a syrupy product with some of the properties of sugars.

We give below Baeyer's suggestion in a translation of his own words.

"The general assumption in regard to the formation in the plant of sugars and related bodies, is that in the green parts carbon dioxide under the action of light is reduced and by subsequent synthesis transformed to sugar. Intermediate steps have been sought in organic acids: formic acid, oxalic acid, tartaric acid, which can be regarded as reduction products of carbon dioxide. According to this opinion, at those times when the green parts of the plant are most strongly subjected to the action of the sun's rays, a strong accumulation of acids should take place, and these should then gradually give place to sugar. As far as I know this has never been observed, and when it is remembered that in the plant sugars and their anhydrides are formed under all circumstances, whereas the presence of acids varies according to the kind of plant, the particular part of it and its age, then the opinion already often put forward, that the sugar is formed directly from the carbon dioxide, increases in probability.

The discovery of Butlerow provides the key, and one may indeed wonder that so far it has been so little utilised by plant physiologists. The similarity which exists between the blood pigment and the chlorophyll of the plant, has often been referred to; it is also probable that chlorophyll as well as hæmoglobin, binds carbon dioxide. Now when sunlight strikes chlorophyll which is surrounded by CO_2 , the carbon dioxide appears to undergo the same dissociation, oxygen escapes, and carbon monoxide remains bound to the chlorophyll. The simplest reduction of carbon monoxide is that to the aldehyde of formic acid; it only requires to take up hydrogen,



This aldehyde is then transformed under the influence of the cell contents as well as by alkalies, into sugar. As a matter of fact it would be difficult, according to the other opinion, by a successive synthesis, to reach the goal so easily! Glycerin could be formed by the condensation of three molecules, and the subsequent reduction of the glyceric aldehyde so formed.

The formation of sugar in a more complicated way is not hereby excluded, and it could very well be possible that plant acids under certain circumstances are transformed into this substance, which in a thousand different forms helps to build up the body of the plant.

In what manner the cell content acts in order to effect the condensation of formaldehyde cannot be concluded beforehand, but one can assume that the sugar formed remains bound with it, and later, according to circumstances, splits off into carbohydrate, sugar, starch or glucoside. This is exhibited at least in the life-history of the slime fungi in which at a certain stage, from a mass similar to the cell content, a great quantity of cellulose is suddenly differentiated. In this connection it would be very interesting to examine chemically the slime fungi in various periods of their life, and determine whether they contain free sugar or free anhydrides, or whether from the plasmodium sugar or cellulose could be split off in the same way that this takes place in the natural process of development."

The experimental evidence which has been adduced in support of Baeyer's hypothesis is not of much interest, and in most cases an unjustifiable parallel is drawn between experiments carried out "in vitro" and processes in the cell. So long as our knowledge of the heterogeneous system in which these latter take place is so

incomplete, it is impossible to draw conclusions from experiments in which the conditions are clearly so different.

The experiments in relation to Baeyer's hypothesis fall into three groups:—

1. Experiments on the formation of formaldehyde in (a) systems containing carbon-dioxide and water, (b) systems containing carbon-dioxide, water and chlorophyll, (c) leaves.

2. Experiments on the formation of sugars from formaldehyde.

3. Feeding experiments with formaldehyde.

1. (a) The production of formaldehyde from carbon dioxide and water in the absence of chlorophyll certainly seems possible under certain conditions. For instance, Fenton (1907) and D. Berthelot and Gaudechon (1910) have each succeeded in reducing carbon-dioxide to formaldehyde under appropriate conditions, but there is no evidence that these conditions are in the least comparable to those in the plant. References to further work in this connection will be found in the paper by Spoehr already cited, in which this side of the question is critically dealt with.

- (b) Experiments dealing with the photochemical production of formaldehyde from systems containing carbon dioxide, water and chlorophyll have all been made with crude chlorophyll, and in most cases oxygen has not been removed from the system. In order to repeat these experiments critically we ourselves extracted some pure chlorophyll ($a + b$). The results obtained in the experiments made with this pure chlorophyll are recorded in a paper by Jörgensen and Kidd (1916). It was found that the production of formaldehyde was always due to the oxidation of chlorophyll. In systems containing only carbon dioxide, water and chlorophyll no formaldehyde is produced.

- (c) Pollacci (1899—1907), Grafe (1906), Kimpflin (1907) and R. J. H. Gibson (1908) contend that formaldehyde can be identified in leaves after illumination, while Curtius and Franzen (1912) contend that other aldehydes are produced, *e.g.*, α β hexylene aldehyde, but in view of the critical experiments of Fincke (1913) and Spoehr (1913) it is clear that under various conditions a large number of substances in the plant will produce aldehydes. Thus experiments of this type do not give support to the formaldehyde hypothesis.

2. In view of the experiments of Butlerow (1861), Fischer (1888, 1889) and Loew (1889), and above all Nef (1910, 1913) it

seems certain that various monosaccharides can be produced from formaldehyde under certain conditions. But these conditions, generally high temperature and alkaline medium, are not the same as those existing in the plant, so that it is impossible to argue from these experiments "in vitro" as to the possible condensation of formaldehyde to sugar in the leaf. Nor do we know of any photochemical or enzymatic reactions which could bring about this change.

3. It has been urged that carbon assimilation should proceed in absence of carbon dioxide if an intermediate product were given as nutrient. Thus Loew (1889) and Bokorny (1888-1911) insist that *Spirogyra* in absence of carbon dioxide, but in presence of the sodium bisulphite compound with formaldehyde can form starch, while Grafe (1909, 1911) and Miss Baker (1913) have urged that plants can so utilise gaseous formaldehyde itself if this is present in the air in a concentration sufficiently low to prevent toxic effects. This only takes place in the light; in the dark formaldehyde is toxic. Spoehr established that formaldehyde vapour mixed with air is quickly oxidised to formic acid in sunlight, so that Grafe's and Miss Baker's experiments could only be used in favour of a formic acid theory of assimilation.

Moreover, the utilisation of a substance by the leaf is no proof that that substance is an intermediate product in carbon assimilation, as we know of several substances which can be utilised by the plant, such as glycerine and sugars not normally found in the leaf, but which nevertheless are not generally supposed to be intermediate products.

Again it has not been found possible to utilise carbon-monoxide in assimilation, as has been shown for example by de Saussure (1804), Boussingault (1868) and Krashéninnikoff (1909).

Thus it is seen, as Spoehr expresses it, that Baeyer's hypothesis, "though alluring on account of its simplicity, is by no means as well established as many writers on the subject would have us believe." Indeed it seems to us that the words of Sachs written 35 years ago (1882, 1887) are as applicable now as on the day when they were written; "whether it is right to claim, with Bertlelot and Kekulé, formic acid or some other member of the formyl group as the first product of assimilation, on account of its simple constitution, I hold as at least very questionable; and it has hitherto been proved by nothing."

C. SUGGESTION OF VAN'T HOFF.

Baeyer's hypothesis is based on the synthesis of carbohydrates in the laboratory by ordinary chemical processes. It is difficult, as we have pointed out, to imagine that the laboratory conditions required for this synthesis should be comparable with those in the plant. Much more stimulating and interesting therefore, is the suggestion of van't Hoff that the reversible enzyme action is a characteristic of many reactions in the plant. It is not clear from the few remarks of van't Hoff in what manner he thought the photochemical reaction and synthetic enzyme reaction should co-operate in the production of carbohydrates. His main interest appears to have centred on the problem as to the substances from which the main products of assimilation could have been synthesized by enzyme reaction. So for instance at a lecture in Düsseldorf in 1898, he said "It was pointed out by Tammann that under the action of emulsin, amygdalin is only partially split and that this hydrolysis proceeds further if the products are removed. Perhaps if he had added a further amount of products of hydrolysis, he might have succeeded in synthesizing amygdalin. Duclaux put forward transformation formulæ, which again suggest the attainment of an equilibrium, and Hill seems to have effected the synthesis of maltose from glucose by means of a yeast enzyme. Unless a ferment undergoes alteration of some kind during its period of activity, it follows, on theoretical grounds, that a condition of equilibrium and not one of total change must be brought about, and that therefore the opposite reaction must be induced. *We are indeed justified in asking the question, whether (by application of the theory of equilibrium), under the influence of zymase and by exceeding a certain limiting opposing pressure of carbon dioxide, glucose might not be formed from alcohol and carbon dioxide, and moreover whether trypsin may not be able, under conditions prescribed by the theory of equilibrium, to form protein from the products of the hydrolysis, which it brings about under other conditions.*" (Bayliss' translation, 1914; the italics are our own.)

It is to be regretted that this suggestion has not attracted the attention of plant physiologists as work on the lines indicated by van't Hoff's suggestion, would at least have been likely to result in lasting contributions to our knowledge of plant processes. Such work is of course considerably more difficult than the carrying out of qualitative tests for formaldehyde, which constitutes the bulk of the work done on behalf of Baeyer's hypothesis.

The subject is clearly one which interested van't Hoff deeply, as is seen from his letters and diary (Cohen, 1912), and he intended to subject the problems to an extensive investigation. Bad health, and finally death, prevented him from carrying out this project, and we only possess from his hands two papers on the subject (1909, 1910) entitled "On Synthetic Enzyme Action," neither of which is of interest here.

Van't Hoff's suggestion obtains a new interest in view of Willstätter's discovery that chlorophyll is a double ester of two primary alcohols, and that leaves contain an enzyme which can effect hydrolysis or alcoholysis of chlorophyll, and can also synthesize chlorophyll from phytol and chlorophyllid (Willstätter and Stoll, 1911, 1913). Unfortunately, as we have pointed out earlier, Willstätter's contention that the amount of pigment is not altered during assimilation, only holds for the chromogen complex, and provides no information as regards the alcohol groups. Therefore we have no indication whether chlorophyllase or the alcohol groups play any part in the processes of carbon assimilation.

D. SUGGESTION OF SIEGFRIED.

Siegfried (1905) worked on the action of carbon dioxide on amino-acids and proteins, and came to the conclusion that definite compounds, carbaminic acids and carbaminates are produced. Thus he says that his results "appear to justify the assumption that where carbon dioxide meets protein in the animal organism, carbon dioxide is fixed organically, and that the compounds so produced dissociate again with evolution of carbon dioxide." After discussing the bearing of this conclusion on various processes in animal physiology, such as blood processes, and the working of muscle, he concludes: "Finally, plant physiology also will have to concern itself with this question. Where there is chlorophyll there is also protoplasm. If by the intake of carbon dioxide by the plant carbamino groups are formed, the intake of carbon dioxide will be accelerated. Instead of, or along with the question, how is carbon dioxide reduced, the question must be solved, how are carbon acids reduced."

It will be seen that we have here a suggestion in regard to the processes of carbon assimilation which differs markedly from the Baeyer hypothesis. It is generally assumed that in the first stage of the assimilatory process the carbon dioxide takes part in

a photochemical reaction; in Siegfried's view on the other hand the first stage of carbon assimilation is a purely chemical process, and the photochemical reaction occurs in a complex carbon compound. This suggestion of Siegfried's has been as completely neglected by plant physiologists as that of van't Hoff, although it offers possibilities of connecting carbon assimilation with nitrogen assimilation in a way which is not possible on the Baeyer hypothesis.

Further interest in Siegfried's suggestion should result from the extensive researches of Ciamician and Silber (1901-1915) on the photochemical reactions in complex organic compounds. Willstätter and Stoll (1915b-d) seem unaware of the work of Siegfried, but express almost identically the same view in regard to the accumulation of carbon dioxide in the protoplasm by means of proteins.

E. THEORIES OF WILLSTÄTTER.

It cannot be said that the development of plant physiology during the last hundred years has been very rapid, nor is the position which it occupies among other branches of botanical science worthy of its importance. This is no doubt largely due to lack of knowledge of the fundamental sciences, physics and chemistry, which must form the basis of all science which is not merely cataloguing or descriptive. As a consequence of this whenever chemists have put forward contributions to the theory of plant processes, their statements have usually been accepted by botanists without reserve. That this has been much to the detriment of plant physiology is evident from a survey of the history of the subject. It is only to be expected that theories of pure chemists on plant physiological subjects should be misleading, when one considers how infinitely more complex are the conditions in the plant compared with the moderately simple laboratory conditions of ordinary chemical experiments. This was true sixty or seventy years ago, and it is true to-day.

Willstätter, whose brilliant chemical work has been, and probably will be in the future, of so much value to plant physiology, has, like the eminent chemists Liebig and Baeyer before him, ventured to put forward theoretical views on the processes of carbon assimilation. Below we give a translation of the first instalment of his theory published in 1906 (p. 64) under the sub-heading of "The Life of the Plant."

"Plants and animals live by means of the catalytic action of

metals, which they contain in the form of complex organic compounds. They differ chemically by the nature and function of the metal. The life of chlorophyll-containing plants is mainly synthesizing. While biology so far has been incapable of giving an explanation, the proof of the presence of magnesium in chlorophyll from all classes of plants allows the conclusion to be drawn that the assimilation of carbon dioxide is a reaction of the basic metal magnesium, which as well known exhibits great power of combination in complex organic molecules. The intake of carbon dioxide is probably a process similar to the Grignard synthesis. The disintegrating (abbauende) life of blood-containing animals requires for the oxidation of organic substances a carrier (Überträger), particularly iron, which, perhaps, on account of its oxidisability, combines loosely with the oxygen and transports it to a series of comparatively unstable compounds. Besides along these main roads natural development along less important roads and blind alleys may have succeeded in the formation of organisms which live by the action of other metals, *e.g.*, copper, and which have shown themselves less capable of evolution.

It is thus seen that there are essentially two kinds of life, which develop along parallel lines of evolution: synthesizing life with magnesium, and disintegrating life with iron, *i.e.*, reducing life and oxidising life."

We have given a translation of Willstätter's views on life in full. Plant physiologists will probably appreciate them without any comment from us. But we may draw attention to the fact that some people regard iron as a synthesizing agent in life, for instance, B. Moore (1914), who has elaborated a theory of carbon assimilation on this view. We do not deal at length with this theory as it involves the formaldehyde hypothesis, and is open to all the criticisms that may be levelled against that hypothesis and a good many more. Moore, besides attempting to explain life as it is at present, utilises his hypothesis for speculation on the origin of life. It may be well to keep in mind the remarks of Darwin in a letter written in 1863, "It is mere rubbish, thinking at present of the origin of life; one might as well think of the origin of matter." (See Darwin, 1902, p. 257.)

Willstätter, it will be observed, attempts to utilise the work of Grignard in justification of the part he attributes to magnesium in his theory. Grignard however rightly points out how very different are the conditions in any Grignard synthesis from those in the plant.

We give below some remarks of Grignard (1913) on this subject.

“ Avec cette extraordinaire faculté d'adaptation aux molécules chimiques les plus diverses, le magnésium ne serait-il pas capable de jouer un rôle très actif dans les synthèses naturelles de la matière organisée ?

Willstätter a, en effet, reconnu que ce métal s'accumulait, pour ainsi dire, dans une substance douée d'une activité catalytique considérable, la chlorophylle.

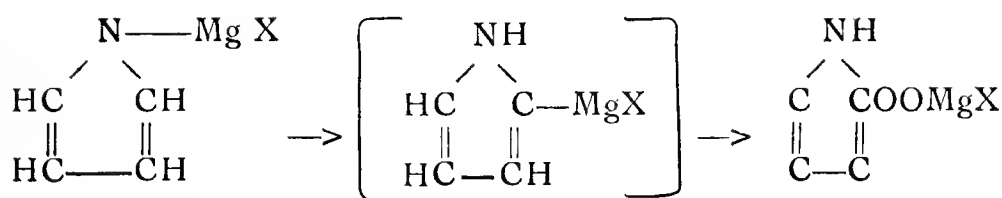
Il a isolé des chlorophylles les plus diverses des combinaisons contenant jusqu'à 3.5% de magnésie. Et il a conclu de ses études qu'il doit se former des combinaisons analogues aux organomagnésiens et que l'absorption du gaz carbonique par la chlorophylle serait tout à fait analogue à une réaction de Grignard. Il est arrivé ainsi à comparer la chlorophylle des feuilles à l'hémoglobine des animaux et à penser qu'il y aurait pour la matière vivante deux cycles de transformation: la vie de synthèse avec l'aide du magnésium et la vie d'oxydation avec l'aide du fer.

Il reste cependant à trouver sous quelle forme le magnésium peut s'approprier à ces réactions, dans un pareil milieu, si différent de celui où nous sommes habitués à le voir triompher. Ce sera le problème de demain.”

A further instalment of Willstätter's theory appears in his book (1913, pp. 23-25), which again we give in the form of a translation of Willstätter's own words.

“ The rôle of magnesium can be imagined to be the same as in those organo-magnesium compounds discovered by Barbier and Grignard which have attained such importance in organic synthesis on account of their reactive properties. Already in our first communication (1906) on the analysis of chlorophyll a parallel is drawn between the latter and the Grignard compounds. The parallel appeared to be inaccurate and met with contradiction because it took no notice of the difference between the binding of a metal to carbon in the ordinary organo-magnesium compounds, and the substitution with nitrogen in chlorophyll. But we do not consider this difference either distinct or characteristic.

Since our publication, B. Oddo has carried out important investigations on pyrrole magnesium iodide which reacts with carbon dioxide and with acid chlorides resulting in the formation of α substituted pyrroles, α carbopyrrole acid, and alkylpyrrolketone. Probably the N-magnesium derivative is first formed, and this is either transformed into the α -magnesium compound, or reacts as such, *e.g.*



The pyrrole magnesium derivatives have thus—analogue with sodiumacetic ester—behaved like any Grignard body with binding of the metal to carbon.

Chlorophyll can be regarded as of the same class of organo-magnesium compounds, and it seems unjustified to draw a sharp line between magnesium phenyl iodide, pyrrole magnesium iodide and chlorophyll, only chlorophyll is characterised by a greater stability of magnesium towards water than the ordinary organo-magnesium compounds on account of the complex binding of the metal.

This comparison does not require that the pigment in the process of assimilation should take the carbon dioxide into its molecule. This can be prevented by substitution in the magnesium-carrying pyrrole nuclei. Rather the function of chlorophyll may be imagined thus: that the carbon dioxide is attracted by the affinity of the magnesium compounds, and that its reduction is effected by the chlorophyll component a in the process which uses the absorbed light energy. Chlorophyll a is hereby oxidised to chlorophyll b, and this is again transformed to the first component with evolution of oxygen. Between the two components an equilibrium condition is obtained.

It is possible that this evolution of oxygen either takes place direct or that the yellow pigments, carotin and xanthophyll take part in the re-formation of chlorophyll a. As the yellow pigments constantly accompany the green pigments in the chloroplasts, it is probable that they have a function. Perhaps this is to regulate the ratio of the chlorophyll components, perhaps by the withdrawal of oxygen from chlorophyll b by carotin, this oxygen being then evolved from xanthophyll by means of the action of an enzyme."

It will be seen that Willstätter here first defends his conception of chlorophyll as a Grignard body. In spite of his elaboration of this conception, it appears to us that his arguments amount to this: In the Grignard syntheses a good many curious things may happen. Carbon assimilation is a curious process which involves the complex organic magnesium compound chlorophyll. Why should not carbon assimilation be a Grignard synthesis

The second part of his theoretical consideration is more interesting, as for the first time we have a suggestion which involves the presence of two different chlorophylls. Although this fact was brought out by the work of Stokes, all subsequent theorists avoided the difficulty by neglecting it. As Willstätter insists that the absolute value of the green pigments and the ratio between them remains constant,¹ there must be a mechanism which keeps the system in equilibrium. The main agency in this, Willstätter suggests, is the yellow pigments assisted by suitable enzymes.

Willstätter's conception of the chlorophyll apparatus as constituting a system in dynamic equilibrium is of course very interesting. However, as long as our knowledge of photochemical reactions and enzyme reactions in the chloroplasts is as imperfect as it is at present, this theory of Willstätter's cannot be accepted as more than a suggestion.

Finally we shall consider the theories expounded by Willstätter in his latest publications (1915, b-d). One of the suggestions put forward in these papers we have already mentioned (Chapter IV, Section E), namely, the reasonable suggestion that carbon assimilation consists of a photochemical process and an enzymatic process. This conclusion was derived from plant physiological experiments with leaves in various conditions.

Further work with isolated chlorophyll was performed in support of his elaboration of the theory, but it is not clear whether this most recent theory replaces the earlier one we have already dealt with, or whether it is intended to supplement it. At least no mention is any longer made of chlorophyll as a Grignard synthesizing agent, nor is any account taken of the *two* chlorophylls, nor of the yellow pigments. Willstätter considers now that a dissociable compound of chlorophyll and carbon dioxide is formed, but as it is formed in the dark he assumes that the function of light is simply to produce an isomer of higher energy content. This assumption carries in its train a number of equally wild speculations which the reader will find in the translation we give below of the summary of the theory.

"As it is seen from the above, the entrance of carbon dioxide into the chloroplast takes place by means of an absorbing substance. The apparatus acts as a carbon dioxide accumulator, as it brings

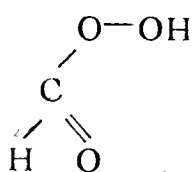
¹ See Chapter II, Section F. Further figures in support of this statement are given by Willstätter and Stoll (1915 b, p. 336) for strongly assimilating leaves under artificial conditions.

the carbon dioxide of the air to a greater concentration, on account of its property to take up more carbon dioxide at lower temperature suited to increase of assimilation under natural conditions. The carbon dioxide wanders on to the place of smallest carbon dioxide pressure.

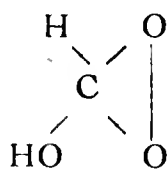
The real assimilation process we can differentiate into several sub-processes. Chlorophyll takes up carbon dioxide and at the same time forms a dissociable compound. One must suppose that this compound takes up light energy, and thereby undergoes rearrangement into an isomer of greater energy content, which is suited for its own disintegration. A transformation product of carbonic acid which can be split off enzymatically with loss of energy, must be imagined as intermediate product, as the observation recorded in the first chapter makes it very probable that a part of the assimilation process is of enzymatic nature.

There is only known one isomer of carbonic acid of peroxide nature to which could be ascribed the rôle of intermediate product, performic acid, obtained in solution by J. d'Ans and W. Frey, which easily dissociates into carbon dioxide and water. In the assimilation process one must of course imagine another way of dissociation of the intermediate product, namely, its disintegration with evolution of oxygen.

As for performic acid, various structural formulæ can be considered



and

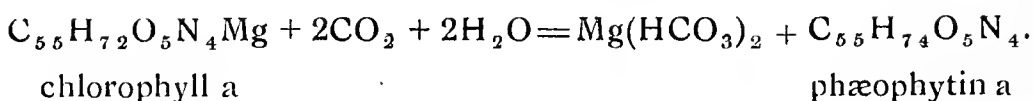


Formylhydroperoxide.

Formaldehydeperoxide.

so it is quite possible that the intermediate product of photosynthesis bound to chlorophyll is a peroxide other than the known substance, performic acid."

In our opinion this latter part of Willstätter's theory, which is based on his experiments with chlorophyll sols and carbon dioxide, is due to premature and incorrect interpretation of his experimental results. These, briefly, are as follows. In a system consisting of a chlorophyll sol and carbon dioxide, a certain amount of carbon dioxide will be absorbed by the water constituting the dispersion medium, and a further quantity will be used in the production of phæophytin according to the equation



but he finds more carbon dioxide is absorbed than can be accounted for by these two processes. For example, 0.505 g. chlorophyll (a + b) was used in 104.02 c.c. water. This absorbed 184.45 c.c. carbon dioxide at 0°C and 747.3 mm. partial pressure, *i.e.*, 6.45 c.c. more than could be accounted for by the water. This is equivalent to 12.6 mg. Of this 7 mg. would be used in the formation of phæophytin according to the equation given above; there thus remains 5.6 mg. unaccounted for.

We do not see any necessity to introduce a mystical and purely hypothetical peroxide in order to explain this result. Indeed, the assumption of the formation of such a peroxide is purely gratuitous in view of the fact that we have absolutely no information in regard to the behaviour of carbon dioxide towards the ester groups of the chlorophyll molecule.¹

From his experiments on the absorption of carbon dioxide in the dark by living leaves and leaf powder, Willstätter concludes that in the leaf there is a mechanism for absorbing carbon dioxide as the leaves and leaf powder absorb many times as much carbon dioxide as can be explained as due to absorption by the chlorophyll. To explain this he puts forward exactly the same hypothesis that Siegfried had propounded ten years before, without, however, making any reference to Siegfried. It may be interesting to compare with Siegfried's results already cited, the remark of Willstätter (1915 b, p. 345) "It is possible that in the absorption phenomenon described, carbamino compounds of amino acids or of proteins are formed. Preparation work is here presented with a new problem." This last sentence suggests that Willstätter is unaware of Siegfried's work.

¹ It is interesting to note that the solubility of carbon dioxide in some alcohols and esters is much greater than in water, see *s.g.*, Just (1901).

CHAPTER VIII.

Concluding Remarks.

In the preceding pages we have attempted to give the outlines of one of the most fundamental problems of plant physiology. The subject has been attacked from many different points of view and by many different methods, but in our opinion the main interest is not centred in the achievements of any individual investigator. What, in our opinion, is the most important aspect which presents itself in reviewing the facts obtained in recent investigations on carbon assimilation, is the prospect of the development of a new phase of science. This is the prospect that plant physiology is developing into an exact science, utilising the experiences of the fundamental sciences, physics and chemistry, but nevertheless a science, exact and independent, with its own working principles and methods, directing and stimulating the development of the applied sciences, agriculture and horticulture. No prophetic vision is needed to foretell that developments in agriculture and horticulture will follow development in plant physiology as great as those which were produced by physics and chemistry in engineering and other technical sciences.

But such development can only take place if we learn from the past what are likely to be the limitations to successful development.

The present state of the subject is the result of a number of independent investigations, the bearing of which on one another is rather accidental than designed, and in this lack of co-ordination is to be found a reason for the slow development of the subject hitherto. It is clear that the only way to attain a reasonable rate of progress is to institute a much closer and more intimate co-operation between scientific workers attacking the same problems from different points of view and by different methods.

It is generally desirable in a review of this nature to conclude with a brief summary of the present position of the subject. In the case of carbon assimilation it seems to us that it is not so much the complete array of experimental facts obtained in the various researches which is of importance, but the general principles which become clear from a consideration of the whole subject. This is especially so as the subject is in a more or less mobile condition and development and ever-widening scope must follow along sound lines of work based on the principles of the subject.

Blackman has brought out the important relation between environmental factors and carbon assimilation, and has formulated the principle of limiting factors in regard to their co-operation. However, absolute rules cannot be made as to amount of assimilation under any definite environment, owing to the complexity introduced by the existence of unknown internal factors. Willstätter has attempted to analyse the internal factors, and has brought proof that chlorophyll is not the only internal factor, though what other internal factors there are Willstätter's work does not show. Future work will have to investigate the inter-relation between the internal factors as well as the co-operation between the internal and external factors.

However, the internal factors operative at any moment are a product of hereditary factors and environmental factors. It seems likely that an application of the principles of genetics may prove helpful in the analysis of internal factors in assimilation, and this application may give a method for controlling some internal factors.

The aim, at present, of investigations on carbon assimilation is to be able to tell the assimilatory power of a plant with a known history as regards environmental and hereditary factors when it is placed in a known environment. Then it becomes of industrial importance to discover how environmental factors can be modified so as to give the maximum assimilation in relation to the inherited internal factors.

We should like to emphasize that the popular idea that under natural conditions any particular factor, as for instance, light, is nearly always in excess, while some other factor, as for instance carbon dioxide, is nearly always limiting, is not justified. The power of the plant to utilise any environmental factor must undergo diurnal and seasonal variations depending on the interplay of the other factors.

For instance the environmental factors, radiation and temperature, undergo daily and seasonal variations while although in regard to the carbon dioxide supply not much information is to be had, undoubtedly considerable variations occur (see, for example, Krantz, 1909). In this connection may be mentioned the work of Kraus (1911) who successfully shows how great may be the variations in environment over a very small area.

The importance of work on carbon assimilation depends not merely on its value in plant physiology and on its application in agriculture, but also, as we have emphasized in our introductory chapter, for the utilisation of radiant energy. For this reason in

the future plant physiology will acquire the co-operation of photochemists, who already take an interest in the work of the plant physiologist. It is encouraging to find an appreciation of plant physiology by the photochemist Plotnikow (1910), whose words on this subject we may quote in conclusion. "From this one realises how much material, work and patience is required for this problem. But let us hope that this will prove no obstacle for further investigations in this field. Labour loving scientists should not lose heart for penetrating further along the path so far prepared.

A brilliant success must finally crown their labour."



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ERRATA.

- Vol. XIV. p. 241, line 34, for "Gibson" read "A. H. Gibson (1913)."
- „ p. 245, lines 32, 36, for "benzol" read "benzene"
- „ p. 285, scheme, for "phæophytin" read "phæophytin a"
- „ p. 289, line 5, delete "in petrol ether"
- Vol. XV. p. 96, line 16, for "1902" read "1899"
- „ p. 145, legend of Fig. 7, for "Krogh" read "Ege and Krogh"
- „ p. 215, lines 12, 13, delete "glucose resulting from the hydrolysis of"

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THE SYRPHID VISITORS TO CERTAIN FLOWERS.

BY E. & H. DRABBLE.

DURING the year 1916 much attention was given by the writers to the flowers visited by the Syrphidæ, a group of the Diptera. Our observations were made in several parts of England, principally in Derbyshire, Middlesex and Hertfordshire, and they supplement rather considerably the information given by Knuth ("Handbook of Flower Pollination," English Edition, Oxford, 1906-09), by Willis and Burkill ("Flowers and Insects in Great Britain," Annals of Botany ix., 1895), by Lord Avebury, and by many other writers whose works we have consulted.

The Syrphidæ, popularly known as the "hover flies," are Cyclorrhaphous Diptera with muscid type of venation, but with long anal cell, closed subapical cell, and a vena spuria. They are generally brilliantly coloured flies, which hover—particularly the males—in bright sunlight, suddenly darting from side to side, and again hovering, and many are very difficult to "net" on account of their extraordinarily rapid movements.

The larvæ of many species feed on Aphides; such are *Pipizella*, *Melanostoma*, *Catabomba*, *Syrphus*, *Sphærophorea* spp. and *Baccha*. Others, such as *Cheilosia* and *Platycheirus* spp., are found in fungi. Others again occur in decaying vegetable matter and cowdung; of these may be mentioned *Leiogaster*, *Platycheirus* spp., *Rhingia*, *Eristalis*, *Myiatropa*, *Helophilus* and *Syritta*. In the sap of diseased trees have been found the larvæ of *Xylota* and *Chrysochlamys*, while those of *Volucella* seem to be confined to the nests of wild bees.

The imagines of nearly all Syrphids are attracted by flowers, particularly by those with plentiful pollen and with exposed or slightly concealed nectar. Accordingly they play a very large part in pollination, visiting the flowers both for pollen, which they eat, and for nectar.

It is not necessary to describe the structure of the mouth parts, as a sufficient account of them may be found in any good text-book of entomology, such as those of Sharp ("Cambridge

Natural History") and Packard, but it may be mentioned that the extended "trunk" differs greatly in length in different species. It is stated that in *Rhingia* it may be as long as 11-12 mm., and in *Syrphus balteatus* as short as 2 mm.

Knuth mentions *Circaea lutetiana*, *Veronica Chamædrys* and *V. Beccabunga* as typical "hover-fly" flowers, but our observations shew, that although these may be visited frequently, many other flowers, quite differently adapted are much more generally frequented by these flies. Knuth also says that the Syrphidæ are specially attracted by "social flowers." They certainly do visit such flowers freely, for example, *Heracleum*, *Leontodon*, *Hieracium*, *Senecio* and *Aster*, but according to our experience they seem to be very generally attracted by flowers that can hardly be described as "social." Thus, we have found *Rubus* to be the most attractive of all the flowers at which we have worked, as regards both species and actual number of individual visits. The comparatively slight reference to *Rubus* in this connection by Knuth is surprising. *Convolvulus arvensis*, an entirely non-social flower, is also freely visited.

As far as our experience goes, the flowers principally visited are as follows:—

Ranunculus acris L., *R. repens* L., *R. Flammula* L. Flowers not aggregated. Visited for nectar and pollen. A nectary lies near the base of each petal and is covered by a small scale. Much pollen is shed from the extrorsely dehiscing anthers, the outermost of which shed their pollen before the stigma is receptive.

Rubus spp. Visited for nectar and pollen. The nectar is produced on a thalamal disc internal to the stamens, and the insect has to push the stamens apart to reach it.

Heracleum Sphondylium L. Flowers aggregated. Visited for nectar, which is produced on an open epigynous disc.

Senecio Jacobæa L., *Aster* spp. (various kinds of Michaelmas daisy), *Chrysanthemum Leucanthemum* L., *Tanacetum vulgare* L., *Leontodon autumnale*, *Hieracium boreale* Fr., *Taraxacum officinale* Weber, *Sonchus asper* Hill. Flowers aggregated. Visited for nectar, which is produced on an epigynous disc and wells up into the tube of the corolla.

Convolvulus arvensis L. Flowers not aggregated. Visited for nectar. Nectaries covered by the broadened bases of the filaments, which leave five narrow passages.

The following table shows the Syrphid visitors to the flowers of fourteen species observed by us during the summer of 1916.

TABLE SHOWING SYRPHID VISITORS TO THE FLOWERS OF
FOURTEEN SPECIES.

	RANUNCULUS ACRIS L.	" REPENS L.	" FLAMMULA L.	RUBUS spp.	HERACLEUM SPHONDYLIIUM L.	SENECIO JACOBÆA L.	ASTER spp.	CHRYSANTHEMUM LEUCANTHEMUM L.	TANACETUM VULGARE L.	LEONTODON AUTUMNALE L.	TARAXACUM OFFICINALE Weber.	SONCHUS ASPER Hill.	HIERACIUM BOREALE Fr.	CONVOVULUS ARVENSIS L.
SYRPHINÆ.														
<i>Pipizella flavitarsis</i> Mg.				x										
<i>Leiogaster metallina</i> F.			x											
<i>Chrysogaster hirtella</i> Lw.			x											
" <i>macquarti</i>			x											
<i>Chilosia pulchripes</i> Lw.				x				x						
" <i>variabilis</i> Pz.					x									
" <i>barbata</i> Lw.	x				x									x
" <i>intonsa</i> Lw.	x				x									x
" <i>albitarsis</i> Mg.	x				x									x
" <i>cynocephala</i> Lw.	x				x				x					x
" <i>impressa</i> Lw.	x													x
<i>Platychirus manicatus</i> Mg.				x	x		x			x	x	x	x	x
" <i>peltatus</i> Mg.				x	x					x				
" <i>scutatus</i> Mg.				x	x					x				
" <i>albimanus</i> F.		x		x	x	x	x			x	x	x	x	
" <i>clypeatus</i> Mg.				x	x									
" <i>angustatus</i> Ztt.				x	x									
<i>Melanostoma mellinum</i> L.		x		x	x		x			x	x	x		x
" <i>scalare</i> F.				x	x									
<i>Leucozona leucorum</i> L.				x										
<i>Ischrosyrphus glaucius</i> L.					x									
" <i>laternarius</i> Müll.					x									
<i>Catabomba pyrastris</i> L.				x						x			x	
<i>Syrphus albobristatus</i> Fln.				x										
" <i>tricinctus</i> Fln.				x										
" <i>lunulatus</i> Mg.				x										
" <i>lineola</i> Ztt.				x										
" <i>vittiger</i> Ztt.					x									
" <i>grossulariæ</i> Mg.				x	x									
" <i>ribesii</i> L.				x	x		x			x			x	
" <i>vitripennis</i> Mg.				x	x	x	x		x	x			x	x
" <i>latifasciatus</i> Mcq.				x										
" <i>corollæ</i> F.					x					x			x	
" <i>luniger</i> Mg.				x	x		x		x	x			x	
" <i>balteatus</i> Drg.				x	x	x	x		x	x			x	x
" <i>cinctellus</i> Ztt.				x	x								x	
" <i>auricollis</i> Mg.				x										
" <i>umbellatarum</i> F.					x									
" <i>compositarum</i> Verr.					x								x	
" <i>labiatarum</i> Verr.				x									x	
<i>Sphaerophorea scripta</i> L.				x	x	x	x			x				
" <i>menthastris</i> L.				x										
" <i>nitidicollis</i> Ztt.										x				

TABLE SHOWING SYRPHID VISITORS TO THE FLOWERS OF
FOURTEEN SPECIES (Continued).

	RANUNCULUS ARCIS L.	" REPENS L.	" FLAMMULA L.	RUBUS spp.	HERACLEUM SPHONDYLIIUM L.	SENECIO JACOBASA L.	ASTER spp.	CHRYSANTHEMUM LEUCANTHEMUM L.	TANACETUM VULGARE L.	LEONTODON AUTUMNALE L.	TARAXACUM OFFICINALE Weber.	SONCHUS ASPER Hill	HIERACIUM BOREALE Fr.	CONVOLVULUS ARVENSIS L.
<i>Baccha elongata</i> F.				x										
<i>Ascia podagrica</i> F.				x						x				
<i>Rhingia campestris</i> Mg.				x										
VOLUCELLINÆ.														
<i>Volucella pellucens</i> L.				x										
ERISTALINÆ.														
<i>Eristalis tenax</i> L.				x	x		x							
" <i>intricarius</i> L.				x										
" <i>arbustorum</i> L.				x	x	x	x		x	x			x	
" <i>pertinax</i> Scop.				x	x	x	x		x	x			x	
<i>Myiatropa florea</i> L.				x			x							
<i>Helophilus pendulus</i> L.				x			x							
MILESINÆ.														
<i>Xylota segnis</i> L.				x										
<i>Syritta pipiens</i> L.	x			x	x								x	
<i>Chrysochlamys cuprea</i> Scop.	x													
Total species observed.	7	2	3	39	29	6	13	1	6	16	3	3	14	10

It is evident from these tables that the plants attracting most species were *Rubus* (39), *Heracleum* (29), *Leontodon* (16), *Hieracium* (14), and *Aster* (13). It is necessary, however, to consider the number of individuals visiting the flowers and not merely the number of species. When this is done it is found that *Rubus* is by far the most attractive, much more so indeed than is indicated by a mere list of species visiting this flower. Next to *Rubus* come *Heracleum Sphondylium* and *Aster*, and far below these in order come *Leontodon* and *Hieracium*. The number of Syrphids visiting *Convolvulus arvensis* was rather unexpected, as it does not strike one as a "hover-fly" flower. We have convinced ourselves, however, that these flies do actually and regularly take the nectar

from *Convolvulus* and do not merely visit it for shelter. On the other hand, the harebell, *Campanula rotundifolia* L., seems to be visited merely for shelter, as we have seen Syrphids in this flower only in the evening or during showers.

We hope to continue our observations on the visits of Diptera during the present year, as it would appear that there is much to be done in this rather neglected branch of ecology.

ON THE PHYSIOLOGY OF PARASITISM.

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AN investigation, begun at the suggestion of Professor V. H. Blackman and carried out under his general supervision, has been in progress for some years, having as its object a study of the physiological phenomena associated with fungal parasitism.¹ With the progress of the research, new branches have been opened, in many cases so inter-related that a definite pronouncement on any one cannot be given till others have been sufficiently explored. Thus publication has not kept pace with the actual progress of the investigation. Many results have been obtained, some which are so well established that they will shortly be published in final form; others which, though in themselves sufficiently established, cannot be dealt with fully until their exact relationships with other

¹ The following papers embodying certain results have been published from time to time in the "Annals of Botany" under the general title of "Studies in the Physiology of Parasitism."

I. W. Brown; "The Action of *Botrytis cinerea*." Ann. Bot., Vol. XXIX, 1915, p. 313.

II. V. H. Blackman and E. J. Welsford; "Infection by *Botrytis cinerea*." Ann. Bot., Vol. XXX, 1916, p. 389.

III. W. Brown; "On the Relation between the Infection Drop and the underlying Host Tissue." Ann. Bot., Vol. XXX, 1916, p. 399.

IV. W. Brown; "On the Distribution of Cytase in Cultures of *Botrytis cinerea*." (In the press) Ann. Bot., Vol. XXXI, 1917.

phenomena, not as yet fully investigated, have been determined; others again which merely serve at present as starting points for further work. It has been thought advisable therefore to publish the following account, which aims at being a résumé of the features already elicited, both published and unpublished, together with a general forecast of the lines which further investigation of the subject may be expected to follow. The present somewhat informal publication has the personal advantage of affording the writer an opportunity to express certain views which appear to him plausible in the light of present knowledge but which may require to be modified as knowledge of the subject extends. It is hoped that it will also enable the general botanical reader to form a more connected picture of the idea and progress of the investigation than may be obtained from the detailed narratives which have appeared and are appearing from time to time elsewhere.

Botrytis cinerea, the fungus which has formed the chief object of study, is a member of that large group of parasitic fungi which are characterised by the fact that they kill the tissue of the host plant in advance of their growth. This species was selected for the present work on account of its ubiquity, the readiness with which it reproduces itself, the rapidity and certainty with which its spores germinate, and the strongly marked features of its parasitic attack. This species is of further interest in that the majority of investigations dealing with the subject have centred round it or its near allies.

It is not proposed to deal in detail with the statements in the literature bearing on this question.¹ Beyond the generally attested fact that such fungi kill in advance of their growth, much of the experimental evidence is of a highly unsatisfactory nature, and the statements put forward by various writers are in many cases contradictory. The primary object of the research was to aim at deciding on certain of these controversial points, and in the first instance to examine the nature of the principle of the fungus which is responsible for the phenomenon of "action in advance." This phenomenon has been known since the time of de Bary (1) to consist essentially of two processes, (a) the solution of the cell wall or at least of certain of its constituents so that the tissue loses its coherence, (b) the killing of the living protoplasmic contents themselves. De Bary believed the phenomenon to be due chiefly to the action of an enzyme, but considered that certain salts (oxalates)

¹ For a detailed criticism, see No. I of the series already cited.

excreted by the fungus played a part in the killing action. Later investigators confirmed the presence of an enzyme in connection with the action and little doubt remained that the action manifested on the cell wall of the host was due to this substance. As regards the killing action of the fungus on the host cell, there has been a progression from the view of de Bary, according to which soluble oxalates may play a part in the effect, to that of Behrens (2), who states that the toxic action is not due to a substance of volatile or enzymatic nature, and finally to that of Smith (3), who ascribes the whole toxic action of the fungus to soluble oxalates.

From a perusal of the literature, it appeared that previous investigators had employed, for the study of the active principle concerned, extracts obtained from comparatively old cultures or from host tissue which had been parasitised by the fungus. Now the actively invading portion of the fungus is essentially of the nature of a young and fresh culture. It may therefore be objected to the former method that extracts of old mycelia do not necessarily bear any close relationship to those of a young vigorous culture, and that in particular the former extracts would contain various products such as the so-called "staling" products which would affect the experiments *in vitro* while they would play no direct part in the phenomenon of parasitic attack. To the extracts obtained from parasitised host tissue, there is, in addition to the foregoing, the further objection that such extracts contain substances derived from the host and not from the fungus at all. It was argued that the presence of the latter would render the experimental results difficult of interpretation, if not entirely nugatory.

I. NATURE OF ACTIVE PRINCIPLE OF FUNGUS.

Preparation of Extract. The aim of the investigation was thus to prepare in the first place an extract from young hyphæ alone, and it was hoped that this extract might prove to be of a sufficiently powerful nature to recommend it for use in this connection. The method which was finally adopted for this purpose is described in detail elsewhere. It consisted essentially in the sowing of a large quantity of spores in a suitable nutrient on horizontal glass plates. A short germination period (ca. 24 hours) was allowed, after which the spores were washed, dried, and ground to a fine powder. The latter was extracted in water, and a clear extract obtained by centrifuging off the débris.

Action of Extract on Plant Tissues.

The method of experiment consisted simply in placing pieces of plant tissue in the extract. In the case of leaves and floral structures, it was found advisable to inject with the extract, as otherwise the action only proceeded slowly from the cut surface inwards (see later). Experiments in which the leaves, etc., were injected with water served to control such injurious effects as might be produced by the process of injection.

In all cases the action of the extract was found to be of a two-fold nature: (1) An action upon the cell walls of the plant, resulting in the complete disorganisation of the cell wall structure, so that the cells separate readily from each other and the cellulose envelope of each individual cell becomes disintegrated; (2) an action upon the living cell itself, resulting in its death.

The former action is shown by the tissue losing its coherence. Thus after a short treatment (about half an hour) with the extract discs of potato or turnip tissue ($\frac{1}{2}$ mm. thick) readily fall to pieces on handling. A similar rotting effect is produced in the case of many foliar and floral structures. In the case of some petals the action is extremely rapid, the injected part becoming limp and obviously disorganised within a few minutes of injection.

A method of quantitatively determining the activity of the extracts employed was elaborated, having as its basis the determination of the time necessary for the decomposition of discs of tissue (potato, turnip) of standard thickness.

The killing action of the extract is shown variously in different cases. In the case of coloured structures it is shown by the escape of the colouring substance from the cells on death. Thus petals of rose, *Viola*, *Gloxinia*, etc., on treatment with the extract rapidly become decolorised. In other cases death of the cells is shown by the development of a colouration which is held in abeyance as long as the cells are alive. Thus when leaves of broad bean are injected with extract, they rapidly become black; when lettuce leaves are similarly treated they become brown. These latter phenomena are due to oxidase reactions which are particular to the plants concerned and have no relation to the fungal extract as such. In the case of colourless tissues, the killing action of the extract can be followed by examining the relation of the affected cells to plasmolysis in hypertonic solutions.

The action of the extract has been tested in this way on a

large number of plant structures. The following general conclusions are drawn:—

Fleshy structures are very generally susceptible to the action of the extract. Among such have been tested the tissue of potato, swede, turnip, radish, apple, cucumber and a number of succulents, (species of stonecrop, *Cotyledon*, etc.).

Petals and floral structures generally are rapidly destroyed. Upwards of thirty species of plants have been tested in this respect, and in all cases a rapid destructive action on the part of the extract has been established. It is highly probable that this behaviour is characteristic of floral structures generally.

Among ordinary foliage leaves a strong action of the extract could be established in some cases, *e.g.*, leaves of broad bean, lettuce, violet, *Petunia*, various species of *Begonia*, etc. In other cases the action was of a much less marked character; with leaves of a leathery or woody texture no definite action could be established in any case.

The extract so far as could be seen had no action whatever on tissues of mosses or liverworts. It was also without action on filaments of algæ and on bacteria.

The detailed features of the action of the extract were followed out in the case of certain tissues (potato, turnip, cucumber, etc.), the plasmolytic criterion of death being here employed. It was shown that the first demonstrable action consisted in the solution of the middle lamella uniting contiguous cells, with the result that coherence of the tissue was destroyed. The attack was, however, not confined to this, and the general body of the cell disintegrated, though complete solution of all constituents did not take place. At a comparatively late stage in the process the cells lost their power of becoming plasmolysed in hypertonic solutions. Thus, of the two manifestations of the action of the extract, the toxic is subsequent to the enzymic.

In dealing with the action of the fungal extract upon tissues, special attention was directed to the post-mortem features evinced. It was found in all cases that these were identical with those induced by the action of the fungus itself. Again, in all cases where a distinct parasitism of the fungus on a particular host could be established, it was found that the tissues of the latter were acted upon in a similar way by the fungal extract; and where the extract was without action (*e.g.* mosses and liverworts), no definite action could be established on the part of the fungus itself. These con-

siderations justify the conclusion that the extract in question is a true representation in essentials of the active principle of the fungus and that therefore the effects of the fungus can be explained on the basis of the properties of the fungal extract.

Behaviour of Fungal Extract to Reagents. An examination of the effect of various substances upon the activity of the extract was carried out. The main results arrived at were as follows:—

Acids and Alkalies. The extract shows a slight acid reaction. When this is slowly diminished by careful addition of alkali, the activity of the extract diminishes, and finally becomes *nil* in the neighbourhood of the neutral point. In alkaline solution the action is in all cases inhibited. On restoring the extract to its original faintly acid condition its activity is restored. With increasing concentrations of acid the activity of the extract again diminishes, the effect of mineral acids being in this respect more marked than that of organic acids. Thus the maximum activity of the extract is shown in faintly acid solution.

Salts. Addition of salts to the extract results in a diminution of activity. The specific retarding action varies with different salts, that of magnesium sulphate, for example, being much greater than that of potassium nitrate.

Substances such as cane sugar and glucose showed a slight retarding effect only when present in high concentration.

Plant Juices. In accordance with the above it was anticipated that plant juices would, on account of the presence in them of various acids, salts, etc., tend to retard the action of the extract. It was therefore of interest to determine how far the resistance of a particular tissue to the action of the extract (and of the fungus itself) could be correlated with the retarding action of its juice upon the activity of the extract. The juices of highly susceptible tissue, —*e.g.*, potato, turnip—and of highly resistant tissue, *e.g.*, liverwort, were compared in this respect. It was found that the retarding action of the latter was of the same dimensions as, and in no respect more marked than that of the former. The conclusion arrived at therefore was that the resistant power of a particular tissue to the action of the fungus (and fungal extract) was not directly related to the chemical nature of its cell sap.

Certain Physical Relationships of Extract.

The results here obtained may be summarised as follows:—

a. Relation to Heating. The activity of the extract, both as

regards its action on the cell walls and on the protoplasmic contents is totally destroyed by a sufficient degree of heating. Deactivation by heat is very rapid at 60°C. At 65°C it is as near as may be instantaneous.

b. Relation to Mechanical Shaking. The extract can be deactivated by agitation, *e.g.*, either by shaking in a closed vessel or by bubbling a stream of air (or nitrogen) through it. The effect is quite marked at ordinary temperatures (15-20°C), but increases very rapidly with rise in temperature.

c. Relation to Diffusion. The extract was submitted to diffusion through a series of graded gelatine membranes prepared in the first instance after the method of Bechhold (4) and later according to the writer's method (5). Dialysates containing various concentrations of the active principle were thus obtained. All the dialysates which showed an action on the cell wall of tissue were also active in killing the cell contents.

From a series of comparative diffusion experiments it was shown that the active principle of the extract possessed a coefficient of diffusion through gelatine comparable with that of dextrin and somewhat greater than that of diastase.

Dialysis of crystalloidal contamination from the active principle was effected by the use of a certain type of collodion thimble (6), as well as by means of gelatine membranes of high gelatin content. The dialysate was found to show no action either on the cell walls of tissue or on the protoplasmic contents.

The dialysate was found to contain a small amount of a calcium salt in solution. The presence of a soluble oxalate in the extract, even in minimal quantity, is thus excluded.

Conclusions as to nature of Active Principle.

The action of the fungus and fungal extract on tissue has been seen to consist of an action on the cell walls together with an action on the living protoplasmic contents. These actions must be assumed *a priori* to be different in nature and to be brought about by different agents. For convenience of discussion we shall therefore speak of the "macerating" and "lethal" principles of the extract, these being responsible respectively for the macerating action (*i.e.*, action on the cell walls) and lethal action (*i.e.*, action on the living protoplasm) of the extract.

It has been shown in the foregoing that the macerating action of the fungal extract can be destroyed in various ways: by heat,

by mechanical agitation, and by neutralisation with alkali. The extracts so deactivated possess no lethal activity whatever. From microscopical investigation it is known that death of the cell takes place at a late stage in the process of disintegration of the cell walls. The latter process is therefore the determining factor of the whole action, that is, there is or is not lethal action according as there is or is not previous macerating action. This dependence of lethal upon macerating activity may be explained according to a variety of hypotheses.

(1) *That there is no distinct lethal substance but that death of the cell follows on account of the physiological derangement caused by the disorganisation of the cell wall.* This hypothesis is unlikely in view of the rapidity with which death of the cells takes place. It is known from plasmolytic studies that the association of cell wall and protoplasm can be broken for many hours without death of the cell ensuing. The rapid effects obtained in the present case cannot, therefore, be ascribed to this cause.

(2) *That the toxic principle is produced by action of the macerating principle on the cell wall, and does not exist, therefore, in the initial extract.* This view must be abandoned in the face of experimental evidence that extract in which a considerable quantity of cell wall has been digested behaves identically in all respects with the original extract.

(3) *That the two actions are due to different substances, but the lethal substance is unable to reach the protoplast until the permeability of the cell wall has been sufficiently increased by the action of the macerating substance.* If there be a lethal as distinct from a macerating substance, it must possess the following characteristics. From the diffusion experiments with gelatine, above mentioned, it must possess a diffusive capacity at least equal to that of the macerating substance; also it cannot be crystalloidal, and in particular it cannot be a soluble oxalate. From the data regarding extracts which have been deactivated in various degrees by heat, mechanical agitation, neutralisation with alkali, it is clear that the lethal substance, if present, must remain in the deactivated extracts (it cannot be assumed to show exactly the same sensitiveness in these respects as the macerating substance). The lethal principle must thus be assumed to be present in deactivated extracts, though it cannot act in virtue of its inability to diffuse through the unaltered cell wall. Killing of the cells should then continue independently of the macerating action after a certain stage is reached, that is when the

permeability of the cell wall has been sufficiently increased. Nevertheless it was found that if the macerating action was stopped (by addition of the requisite amount of alkali), even at a very late stage, the killing effect is strongly retarded. Such an experimental result is not favourable to the present hypothesis.

(4) *That both actions are due to the same substance or group of substances.* This view appears to the writer to be the simplest, and so far as is known, is in agreement with all the experimental facts.

While hypotheses (1) and (2) are in the mind of the writer definitely excluded, a final decision between (3) and (4) cannot at present be given. From the point of view of the progress of the present investigation the question at issue is not of primary importance, as it is sufficient for present purposes that the lethal action of the extract has been shown to be indissolubly connected with its macerating action. On theoretical grounds, however, this question is of very considerable moment. If the last-mentioned hypothesis be confirmed it would appear that we have in the active principle of the fungal extract a protoplasmic toxin which is presumably of an enzymic (*i.e.*, hydrolytic) nature. It is known that the active principle under consideration shows considerable specificity in regard to its behaviour towards cell wall constituents (cell walls of different plants, different constituents of the same cell wall). If such a behaviour could be shown in the case of the corresponding protoplasmic membranes, a practicable study of protoplasmic specificity could be undertaken. The main obstacle to such a study is one of technique. It would be essential to study the action of the extract upon the protoplasmic membrane independently of previous action on the surrounding cell wall, that is it would be necessary to experiment with protoplasts which are naturally naked or with ones which have been made so artificially. As the latter would probably furnish the data of chief interest, the technical difficulties in the way of this investigation are obvious.

CONCLUSIONS ON THE NATURE OF THE FUNGAL EXTRACT.

Whichever hypothesis be accepted as explaining the lethal action of the extract, it is clear in any case that the chemical nature of the cell wall is of fundamental importance in relation to the action of the fungal extract upon the cell. In all cases it has been found that if the cell wall is disintegrated, death of the cell ensues; if the cell wall is not affected, neither are the living contents

of the cell. In other words, the nature of the cell wall affords the key to the resistance of the particular tissue to the action of the fungal extract and therefore also of the fungus. In particular, certain experiments lead to the conclusion that there are important chemical differences between the cell walls of higher plants and those of lower forms, such as liverworts. These considerations point to the desirability of a more complete study of the hemi-cellulose (or pectin?) series of cell wall constituents than has yet been attempted.

General Conclusions on the nature of the Fungal Extract.

At this stage it will add to clearness to summarise the main results obtained.

The action of the fungus on plant tissue may be studied on the basis of the action of the extract employed. The macerating principle of the latter is of the nature of an enzyme and is a colloid of intermediate diffusive capacity. The lethal principle, if different from the former, is also colloidal, with a diffusive capacity at least as great as that of the enzyme. Crystalloids, and in particular, soluble oxalates play no part whatever in the lethal activities of the extract.

II. EXCRETION OF ACTIVE PRINCIPLE BY THE FUNGUS.

From the phenomenon of "action in advance" already alluded to, it is clear that the active principle of the fungus is able to diffuse out of the fungal hypha into the plant tissue. An investigation was carried out with the object of determining to what degree the fungus was able to excrete its active principle into the culture medium. The results of this investigation are chiefly of technical and enzymological interest, and as they are of subsidiary importance in the progress of the investigation except in so far as they serve to indicate how the various contradictory statements on the subject have arisen, they will merely be referred to here. The amount of enzyme present in the culture fluid was found to increase up to about the time at which active growth of the hyphæ ceased, that is, to the time at which "staling" of the culture was reached, after which, the concentration continuously diminished. The nature of the preparation obtained was found to depend also upon the density of sowing and on the nature of the nutrient fluid. Some of the preparations obtained in this way are of a very active nature and contain much higher concentrations of enzyme than the standard extract previously employed. They are, however, less suitable for such a study as that described in Section I of this paper on account

of the presence in them of considerable quantities of impurities derived from the culture fluid.

III. ON THE ENTRANCE OF THE FUNGUS INTO THE HOST TISSUE.

Fungi of the type of *Botrytis cinerea* are able, in favourable circumstances, to attack their host when simply brought in contact with the external surface of the latter. Their entrance is not conditional upon the existence of pre-formed injuries as is the case with so-called "wound" parasites, nor do their germ tubes as a rule enter by way of the stomata or other natural apertures, but penetrate directly through the epidermis. A study of the mechanism of this process of penetration is obviously of fundamental importance.

The dominant view on this question is that due to de Bary (l.c.). In the case of the allied fungus, *Sclerotinia Libertiana*, he stated that the fungus, when placed in contact with the epidermis, possessed the power of killing the underlying cells of the host before any actual penetration of the latter had taken place. In consequence of this an exosmosis of food material takes place on to the epidermis. This serves to promote the growth of the fungus which is now able to penetrate directly into the underlying cells.

In the light of the above view it was important to determine whether the extract employed in the present investigation was able to exert any influence when placed on the outside of the host plant. Such action would consist in the death and disintegration of the underlying epidermal cells, with or without an alteration of the mechanical properties of the cuticle.

These experiments were carried out in very great detail and on a considerable number of plants. Though in a very few cases traces of action by the fungal extract under these conditions could not altogether be discounted, the evidence obtained was overwhelmingly in favour of the opposite view. In the great majority of cases no action whatever on the part of the extract when laid on the surface of plant structures could be demonstrated. The striking nature of this feature may be illustrated by mention of a particular experiment. A fungal extract when laid on a rose petal for twenty-four hours produced no change whatever. After this lapse of time, injection of the same liquid into the same petal produced complete disintegration within half an hour.

It was thus clear that the extract under consideration is unable to penetrate cuticularised tissue and thus cannot explain the killing-before-penetration effect described by de Bary. It was necessary

therefore to examine the early stages of infection by the actual fungus in order to check the statements of de Bary.

De Bary's view necessitates the existence of a toxic substance capable of diffusing through cuticularised membrane. This consideration postulates a considerable diffusive capacity on the part of this substance so that it should become uniformly distributed (or nearly so) throughout the limits of the small drop of nutrient in which the fungal spores are placed on the plant. Thus if such a toxic substance be present, the underlying tissue should be killed as near as may be simultaneously over the whole surface of contact of the drop and the plant. In a large number of cases however it was shown that the first appearances of attack showed a definite pattern within the drop, commencing as a marginal ring and spreading centre-wards. Again, on the same hypothesis, the time for the attack to develop should depend directly on the concentration of spores present, and inversely on the size of the nutrient drop, the larger the latter the longer the time required till an effective concentration of the toxic substance is reached. Nevertheless it was found that within very wide limits the time required for attack to manifest itself was independent of the number of spores present and of the size of the drop. Also, when drops in which infection had just been established were taken up, cleared of spores and tested on fresh pieces of plant tissue, no killing action whatever was demonstrable.

Further convincing evidence on this subject was obtained by use of a conductivity method. Drops of water with or without spores were laid on certain plant structures, and the electrical conductivity of the drops determined from time to time. In the case of both spore-free and spore-containing drops, a gradual and equal increase of conductivity was shown up to a stage when macroscopic discolouration of the underlying tissue in the latter case was observable. The conductivity in the latter case then increased with great rapidity, thus showing that a strong exosmosis was taking place from the host cells in virtue of the action exercised by the fungus. The drops in which this rapid exosmosis had begun when subjected to microscopic examination invariably showed penetration by the fungus to have already taken place. Indeed, in a number of fortunate cases, early stages of penetration could be demonstrated in drops in which the rapid rise of conductivity had not yet begun. Thus it is clear that the rapid exosmosis of soluble cell contents which is necessary according to the de Bary hypothesis to enable

penetration of the cuticle to take place, actually takes place only when penetration has been fully established.

A microscopic investigation (No. II of the series) carried out with the usual technique confirmed these results in every way. It was shown that in the case of the attack of the fungus on leaves of broad bean, the underlying tissue was wholly unaffected up to the time when penetration had actually taken place.

It is clear therefore that the de Bary hypothesis is untenable, at least in the present case, and that *fungi such as Botrytis cinerea exert no influence whatever upon the host tissue until the barrier offered by the outer-cuticularised layers of the latter has been passed.*

The process of penetration is thus conceived as follows:—When spores of *Botrytis cinerea* are placed in a drop of water on the surface of a plant, the sources of energy required to enable them to penetrate the cuticular barrier consist simply of that stored within the spores themselves and of that which diffuses passively from the uninjured plant into the water drop. The phenomenon, therefore, falls to be investigated along three distinct lines: (1) An investigation of the germinating capacity of the fungal spores in pure water and in nutrients. (2) An investigation of the rate of escape of nutrient materials from plant tissue into water placed in contact with its outer surface. (3) An investigation of the physics of cuticular resistance on the one hand and of the power possessed by fungi of penetrating membranes on the other. An investigation of the existence and nature of tropic forces also calls for treatment in this connection. These investigations are still incomplete in detail, but enough work has been carried out to indicate the lines along which advance will be made.

(1) *On the germinating capacity of fungal spores in water and nutrients.*

The necessity for this investigation was only brought home at a comparatively late stage in the research, so that only a limited number of data are yet to hand. The object in view was in the first instance a purely practical one—*viz.*, to control the amount of spore germination for the general experimental work.

The method of experiment consisted in the preparation of spore suspensions in water by means of filtration through fine muslin. The water was purified by repeated centrifuging and renewal until the conductivity of the suspension equalled that of distilled water.

Means of estimating and regulating the density of spore suspension were also devised. The following results are well attested.

With any given lot of spores when sown on glass in pure water the percentage of germination is a function of the concentration of the spores, the higher the concentration of spores present the lower the relative and absolute amount of germination. Thus, with any particular lot the concentration can be increased so that germination is totally inhibited.

When the spores are sown in very dilute nutrient a similar phenomenon is observed, the concentration required to produce inhibition of germination being increased.

The percentage and vigour of germination of spores under a given set of circumstances depends also upon the nature of the spores. The main fact revealed in this connection is that age of spores diminishes capacity for germination. Thus the germination in water of spores from somewhat old cultures can be inhibited by a lower concentration of spores than is the case with spores from a young culture. This process might be described as one of "attenuation" by age. Other methods of attenuation can no doubt be devised—such as, perhaps, the maintenance of the spores under somewhat unfavourable physiological conditions. A thorough examination of these relationships is certain to be of great value in the elaboration of methods for the experimental treatment of the whole subject.

A method was also sketched by which the amount and vigour of germination under any circumstances could be controlled by subjecting the spores to the influence of varying percentages of carbon dioxide in the atmosphere. It was found that the effect of small amounts of nutrient could be neutralised by placing the fungus in the appropriate concentration of carbon dioxide. Thus while germination of a particular sample in pure water could be inhibited by an atmosphere containing 10% carbon dioxide, a pressure of 30% carbon dioxide was necessary to inhibit germination in a certain concentration of turnip extract.

The amount of germination in water can also be controlled by addition of very small amounts of alkaline carbonate. Evidence was also obtained that the effect of slight concentration could be neutralised by the addition of the requisite amount of neutral nutrient solution. The experimental possibilities of this method have not yet been investigated sufficiently fully to allow of further statement.

(2) *On the escape of nutrients from plant tissue into water placed in contact with its outer surface.*

Investigation in this connection has gone so far as to establish that drops of water when placed on the epidermis of plant structures do not remain as such but that substances diffuse out into the drops from the living tissue beneath. The drops may thus come to contain an appreciable amount of nutrient available for the germination of fungal spores in the drops. The method adopted consisted in the laying of drops of distilled water on leaf, flower, etc. structures for definite intervals, these drops being subsequently taken up and added to drops of a spore suspension in pure water. The effect on germination of the drops which had been on plant epidermis were compared with that brought about by drops of distilled water which had lain a similar time in contact with glass. By this means the escape of nutrient substances could readily be detected in the case of certain living plant structures. Considerable exosmosis of such nutrients was shown in the case of petals of *Cereus*, *Phyllocactus*, certain varieties of tulip; intermediate amounts in the case of sweet pea; and small traces only in the case of rose petals, bean leaves, etc. The investigation was also carried on by an conductivity method, with confirmatory results.

The exosmosis of nutrients from tissue could also be demonstrated in special ways. Thus it was found by the spore method that in *Cereus* petals a very considerable exosmosis took place, in *Lilium* petals, on the other hand, very slight exosmosis. It was found that the attack of *Lilium* petals by the fungus could be inhibited by a pressure of 15% carbon dioxide, whereas the latter required to be increased to a pressure of 30% to inhibit attack on *Cereus* petals.

The magnitude of the exosmosis of nutrients may also be measured by noting the difference between the times necessary to produce infection when the spores are sown in nutrient and when the spores are sown in water, the spores in the former case being sown in a concentration sufficient to inhibit germination on glass in pure water. With increasing exosmosis the time difference becomes less and less, and in the case of certain petals (e.g. *Cereus*) may become infinitesimal.

(3) *On the physics of cuticular resistance and the penetrating power of fungi.*

From the fact that the fungal extract has no effect whatever when laid on cuticle, and from evidence obtained in the microscopi-

cal investigation already alluded to, it is clear that the actual process of penetration of the cuticle takes place in a purely mechanical way. In investigating this problem several subsidiary problems arise. Mechanical pressure requires the existence of a fulcrum or attachment of the germ tube to the cuticle. The nature of this attachment, its presence or absence in different fungi requires investigation. Again, evidence has been brought forward that different fungi under the same conditions have different powers of mechanical penetration. A method was devised for testing the penetrative power of fungi. This consisted of the use of a series of gelatine membranes of graded hardness (5). It was found that a membrane of greater hardness was required to prevent the passage of *Botrytis* than was required in the case of *Rhizopus* or *Penicillium*. It is hoped when the details of this method are worked out that the penetrative powers of fungi can be catalogued on the basis of this graded series of gelatine membranes.

The question of penetration cannot satisfactorily be treated without consideration of the tropic factors which may come into play. The researches of Miyoshi (7 and 8) pointed to marked chemotropic influences in this connection, but these have been to a large extent discounted by the later work of Fulton (9). The recent work of Graves (10) has demonstrated the existence of a positive chemotropism exercised by cane sugar and, more especially, certain unknown constituents of turnip juice, together with that of a marked negative chemotropism of the fungus to its own excrete products. In the case of membrane penetration, positive chemotropic forces may conceivably play a part; negative chemotropism to the excrete products of its growth is physically impossible under the circumstances (If anything, there should, on the hypothesis of of such a force, be a tendency for the germ tubes to grow *away* from the epidermis into the spore-free region of the drop). While a positive chemotropism is thus not excluded, experiments have already shown that penetration of membranes may take place under circumstances which exclude such a factor. Fungi have been found to penetrate the following membranes:—

- (1) Membranes of paraffin wax, which were shown by collateral test to be impenetrable to the simplest electrolytes.
- (2) Plant epidermes which had been removed and repeatedly washed till all soluble substances had been removed.
- (3) Gelatine membranes which had been soaked in exactly the same nutrient as the spores were sown in.

In all cases vigorous penetration took place provided the spores were in a condition to germinate.

The view which seems at present most likely to the writer is that the stimulation to penetration is a contact one. It is quite feasible of course that chemical factors may influence the nature and degree of this "thigmotropic" response. It is however premature to dogmatise on this subject as the amount of reliable data available is insufficient for the purpose of generalisation.

General Considerations on Plant Immunity and Resistance.

The general trend of this research has been to show that nothing short of a full physiological investigation of fungal metabolism on the one hand and of at least certain aspects of higher plant metabolism on the other hand must be undertaken if any insight into the problems of resistance and immunity is to be obtained. The extraordinary diversity of the observational data on this subject is sufficient to prove the hopelessness of trying to explain these phenomena on the basis of one or two features of the fungus on one hand and of the parasite on the other. The establishment of parasitism by a particular fungus on a particular plant follows upon a long series of physiological actions on the part of the fungus and simultaneous actions or consequent reactions on the part of the plant. With highly specialised "obligate parasites" the reactions between host and parasite are of a very recondite nature, and as yet there seems no possibility of subjecting them to experimental analysis. The application of the methods of the present research to such cases is thus problematical. In the case however of less specialised forms such as *Botrytis*, the reactions of host and parasite are comparatively simple in virtue of the phenomenon of "action in advance." On this account the host plant can be treated as a special kind of culture medium. Thus the problem entails simply the investigation of the growth of the parasite in a culture medium together with that of the enzymic process by which the living plant is converted into a dead culture medium. Nevertheless though the whole problem can be attacked on the lines of known methods, the phenomena presented are of a complex character, and as has been already emphasised a complete physiological investigation of a large number of processes must be carried out if any advancement is to be made.

A scheme of research adequate to the needs of the problem may best be devised by consideration of the successive stages by

which parasitism on a particular plant becomes established. When fungal spores come in contact with the plant surface, the first desideratum is a sufficiency of water to enable germination to take place. An investigation of hygrometric conditions is thus necessary as determining the presence of this water, and also as preventing at a later stage the spreading of the attack by the drying up of the part affected. In cases where the initial spore germination is dependent on dew formation an investigation of the speed of germination is of great importance. Granted a sufficiency of moisture for germination, the capacity of the particular spores to germinate in the drop falls for consideration. This problem as has been shown entails a two fold investigation, *viz.*, the capacity of the spores to germinate in water in virtue of energy stored within themselves, and the passive exosmosis of food materials into the drop from the underlying plant tissue. The next essential step in the process is that the fungus with the food material available to it should be able to penetrate the cuticle (or, in certain cases, should be able to advance as far as the neighbouring stomata and so into the interior of the plant). The treatment of this problem entails the examination of the intrinsic penetrative power of the fungus on the one hand, and of the mechanical resistance of the cuticle on the other, together with an estimation of possible tropic factors concerned. The difficulty in this regard is one simply of methods of experiment. Once penetration of the cuticle has taken place, the problem becomes simply an enzymological one. If parasitism of the type shown by *Botrytis cinerea* is to be established, the fungus must be able to kill the adjacent host cells, and continue to live in and progress through the dead remains. The problem is therefore one of the action of the fungal principle upon the particular living cells, and of the capacity of the fungus to live and excrete its active principle in the juice of the plant.

In the case of the so-called wound parasites (among which most bacteria would appear to fall) the problem presented is much simpler, as the problems which arise antecedent to the penetration of the cuticle do not come into consideration. Such parasites are thus to be investigated on the lines of the latter part of the scheme sketched above.

The investigation of a number of parasite and host types, according to the scheme advocated in the present paper, would prepare the way for a treatment on physiological lines of the phenomena of resistance and immunity.

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RECENT WORK ON TRANSPIRATION.

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RESARCHES on transpiration in recent years have resulted in the recognition of the complexity of the system involved in the regulation of the rate of water-loss from a plant. It has been realised that the control of water-loss is not a simple process, dependent mainly on stomatal change, but that its regulation is shared by a number of widely different factors, which are nevertheless more or less closely interrelated. Some of these regulating factors may be described as external, pertaining to atmospheric and climatic conditions, such as temperature, atmospheric humidity, and light; whilst others are classed as internal, that is, pertaining to the structure and condition of the plant. Examples of the latter are stomatal aperture and the supply of water to the transpiring leaf cells.

Transpiration being essentially a process of evaporation, external factors may be expected to influence the process in much

¹ Dr. Knight's paper forms a useful summary of work on the current problems connected with transpiration which are being attacked by plant physiologists. It contains some account of the author's own unfinished researches, including the methods he has adopted and some partial results. Under ordinary circumstances it would have been undesirable to publish these, but in view of the temporary removal of the author from research owing to the national crisis it has seemed legitimate to include them.

[EDITOR N.P.]

the same way as they influence the rate of evaporation, other conditions being equal.

Renner (26) has carried out a series of researches on the influence of various external factors on the rate of transpiration, and has emphasised the importance of air movements in connection with the rate of loss of water from the leaf. He found that a wind had a greater effect on the rate of loss from a large leaf than on the rate of loss from a small one, and also that in moving air transpiration is more nearly proportional to leaf area than is the case in still air. He also conceived a "point of saturation" existing somewhere within the tissues of the leaf, *i.e.*, presumably a point of maximum water content of the air. Renner imagined this saturation-point to be sometimes immediately beneath the stomatal pore and sometimes deeper in the mesophyll. When the point of saturation is immediately beneath the stoma the transpiring system may be considered to consist of the pore of the stoma, but when the point of saturation is deeper in the mesophyll, the system consists of the pore plus a length of inter-cellular spaces. In the latter case a factor independent of the stomata is introduced and the part played by the stomata in the control of transpiration is proportionately diminished. From his experiments Renner concluded that in still air the saturation point is immediately below the pore of the stoma, whilst in a wind it is deeper in the mesophyll. Therefore in a wind the importance of the stomata in the control of transpiration is less than in still air. A consideration of the path along which the water vapour must pass from the transpiring mesophyll cells to the outer air, at once shows that the point of maximum water content of the air must be at the surface from which the water is evaporating, and that the water content must diminish towards the outer air. Renner's general conclusions, however, appear to hold good.

The same author (27) has carried out some experiments on the influence of the external conditions occasioned by the nearness of transpiring leaves to each other. He found that the rate of evaporation from vertical surfaces was not appreciably affected by other evaporating surfaces if they were more than two cms. apart. Thus, since external factors which influence the evaporating power of the air also influence the rate of transpiration, it is necessary when dealing with any of the internal factors affecting transpiration, to control or to eliminate the effect of the external factors.

Livingston (19) introduced the conception of "relative transpiration," the use of which was designed to eliminate the influence

of the changing evaporating power of the air. "Relative transpiration is the ratio between the actual transpiration rate and the rate of loss of water from a standard evaporating surface under the same conditions as the plant. The assumption involved in this method is that the rate of the loss of water from the evaporating surface (atmometer) is influenced by the changes of the external conditions in the same manner as, and proportionately to the transpiration rate. It has been pointed out elsewhere that this assumption is not necessarily correct under all conditions (15).

Livingston in 1913 adapted Stahl's cobalt paper method of measuring transpiration rate (20), and by this means controlled the external conditions influencing transpiration. This method with the addition of more recent improvements has been frequently used (1, 2, 3, 22, 30), and appears to be very satisfactory.

Briggs and Shantz (6), in an extensive research conducted under field conditions, have dealt with the external factors affecting transpiration. Their method was to observe the plants through the normal daily cycle without attempting any control of environment. Records were kept of all the external factors, and their individual influence on the transpiration rate was calculated. The results showed that some of these factors were inter related, and also that there existed a factor (probably internal) which had not been taken into consideration. The authors were of the opinion that this factor was either stomatal control or the water supply to the leaf.

The size of the stomatal apertures has long been recognised as a factor concerned in the regulation of the rate of transpiration, but the extent to which this regulatory function is shared by the stomata with other internal conditions of the plant is a question which has been the subject of some controversy. Darwin (7) considered that the stomata play a predominating part in the control of transpiration, whilst Lloyd (23) thought that their importance was small. The question has been dealt with more fully elsewhere by the present writer (12).

The water content of transpiring cells is also a factor which may influence the rate of transpiration. Livingston and Brown (21) concluded that the internal factor, which Livingston (19) had previously suspected to be the cause of the reduction of the transpiring power of plants in the morning hours, was the lack of water in the cells of the leaf. More recently Mrs. Shreve (29) has obtained similar results, and entirely confirmed the work of

Livingston and Brown. This is also in complete accord with the suggestion offered by Briggs and Shantz (see above).

Some workers have investigated the inter relations of the water content of the leaf and the size of the stomatal apertures. Darwin (7, 8) and also Darwin and Pertz (9) found that whilst the wilting of a leaf finally caused stomatal closure, the first effect of loss of water was to produce an opening movement. Lloyd (23) failed to find this preliminary opening while Laidlaw and Knight (16) obtained results similar to those of Darwin and Pertz. Iljin (11) thought that excessive transpiration produced stomatal closure by reducing the osmotic pressure of the guard-cells, changes of water content serving to stimulate the action of enzymes which brought about an alteration in the osmotic substances of the cell. Iljin, however, also states that he found open stomata frequently in wilted plants, and that the rate of loss of water has no effect on the rate at which the stomata close. The first of these statements seems to confirm the idea that the stomata may open as the first consequence of the loss of water.

The direct effect of light on the rate of evaporation from leaf tissues, as distinct from the indirect effect of light on transpiration by the production of stomatal changes, has also been studied. Darwin using a new method (10), found that the mesophyll of a leaf transpired more rapidly in the light than in the dark. His method was to block the stomatal apertures by means of vaseline, and then to put the mesophyll in communication with the outer air by slitting the leaf in several places. Thus any effect of light upon the stomata is prevented from influencing the transpiration rate, since the loss of water takes place through the slits and not through the stomata. The treatment is rather a drastic one but it appears to give consistent results, and Darwin found that the rate of evaporation from leaves treated in the manner described was more than 30% greater in daylight than in darkness. The experiments, however, were conducted under conditions which were not very closely controlled, and it seems desirable to repeat them either in a more constant environment or taking into account the changes in the external conditions.

The question of the effect of light on the transpiring properties of the internal tissues of a plant has more recently been investigated by Mrs. Shreve (28) working with *Opuntia versicolor*. Succulents of this type are found to have a higher transpiring power at night than during the day (Livingston, 17), and Mrs. Shreve's work was directed to discover the reason for this



Fig. 1.



Fig. 2.



Fig. 3.

abnormal state of affairs. Experiments on the rate of transpiration, the rate of absorption, and the changes of stomatal aperture were carried out, and it was found that the stomata were more widely open during the night than during the day. The capacity of the internal tissue to hold water was determined by placing in water, cylinders cut from the fleshy stems and then, by weighing, the maximum amount of water which the tissue would contain was determined. The results showed that these internal cells were capable of holding more water during the day than during the night, that is the resistance offered by the plant to the drying tendencies of the environment is greater by day than by night, presumably owing to the differences of illumination. The author suggests (p. 117) that the abnormal changes may be explained by the alterations of the water-holding capacity of the internal cells. During the day, when the internal tissues have a greater tendency to hold water, the outer cells in the epidermal region have a correspondingly greater difficulty in obtaining water from within to replace that lost by transpiration. The result is a decrease in the water content, and therefore of the turgidity, of the epidermal cells. With the loss of turgidity the stomatal guard-cells collapse, and the size of the pore is diminished. Mrs. Shreve thinks that it is possible to regard the stomatal changes which were observed, as being of sufficient magnitude entirely to account for the unusual cycle of the changes of the transpiring power (p. 115). Thus, if Mrs. Shreve's suggestion is correct, the variations of water-holding capacity, and therefore of water content, become effective indirectly by causing stomatal variations. This is a point of view differing slightly from that of Darwin, and also from that of Livingston and Brown. Darwin (10) thought that changes of illumination had a direct effect on the capacity of the leaf cells to retain water, and that this altered the amount of water passing into the atmosphere from the plant, independent of any other variations brought about by stomatal changes. According to Darwin's theory the effect of light is to increase the rate of transpiration, whilst Mrs. Shreve postulates a lower rate of water loss in the light. It must be borne in mind, however, that in the latter case the plant concerned was one of abnormal behaviour. Livingston and Brown (21) conceived that the change of the evaporating power of the plant tissues was also directly affected by variations in the cell water-content, again independent of any change of stomatal aperture.

Mrs. Shreve's explanation of the phenomena noted in the case of *Opuntia* may suffice for the particular group of plants, but it

must be observed that the theory requires the assumption that the water-retaining properties of the outer regions are different from those of the more deeply seated tissues of the plant. If the water-holding capacities of all parts of the plant were similarly affected by changes of illumination, then the relation between the water-content of the epidermal layer and that of the internal tissues should remain the same throughout the day, other conditions being equal. This, according to Mrs. Shreve's theory, is not the case, so that it is necessary to assume a difference between the effect of light on the epidermal cells and the effect on the internal tissue, in order to explain the difference of behaviour in light and darkness.

In 1913 the present writer, at the suggestion of Professor V. H. Blackman, undertook a series of researches on the various factors concerned in the control of transpiration. The work has been in progress since that date, but as it has now been unavoidably interrupted, it was thought desirable to give some account of the methods in use and to summarise the results obtained.

Attention was first directed to the influence of stomata on transpiration, and the methods of measuring the aperture of stomata were considered. The porometer method of Darwin and Pertz (9) was fixed upon as the most attractive, and a considerable time was devoted to the testing and improvement of the manner of operation of this apparatus. A convenient form of the porometer was devised, and this was described in 1915 (13). In conjunction with Mr. Laidlaw this apparatus was modified to render it self-recording (16), thus obviating the necessity of continuous attention when a continuous record is required. Self-recording porometers had already been described by Balls (4) and by Neilson Jones (25), but these were comparatively elaborate, whilst that devised by Laidlaw and Knight is simple in construction.¹

With the help of these forms of apparatus the porometer method was tested with a view to determining its efficiency. The results were reported in 1916 (14), and the following are the important points which were brought to light.

The pressures used to draw the stream of air through the leaf should be as small as convenient to avoid the possibility of altering

¹ It may be mentioned here that during the greater part of 1916 a new form of the recording apparatus has been in use with very satisfactory results. The principle is that of the Mariotte bottle, as in the apparatus described in the paper cited above, but in the new form the pen of the recorder is actuated by the discharge of the bubble from the end of the air-intake tube, not by the fall of the drop from the end of the dropping-tube as in the earlier apparatus. By this means the apparatus can be used satisfactorily under ordinary conditions without a constant temperature bath or a water jacket.

the size of the pores by deformation of the leaf resulting from suction. The air-stream should be drawn through the leaf only when readings are actually being taken, since some stomata have been shown to be sensitive to the stimulus of the continued passage of air. There is also probably a desiccating action to be feared. Some stomata are sensitive to the shock produced by handling the leaf, and it is therefore advisable to move the plant as little as possible, or to allow it sufficient time to recover from any necessary handling before taking readings. It was also made clear that the resistance offered by the inter-cellular spaces of the leaf to the passage of the air-stream could not be neglected, and a modification of the form of the cups attached to the leaf was found desirable. The experiments also showed that the stomata on different parts of the same leaf behave similarly in their reaction to the changing environment, but the behaviour of stomata on different leaves is not so uniform. It is therefore advisable, when dealing with a shoot, to fix leaf-chambers to several leaves and to take a composite reading from them all, as has already been suggested by Balls. The results of this work showed that with certain modifications and some special precautions, the porometer method gave consistent readings, and it was therefore considered satisfactory.

Experiments were then conducted on the relation of stomatal change, as indicated by porometer readings, to transpiration rate. It was recognised as essential that the influence of the external conditions included in Livingston's "evaporating power of the air," should be either controlled or eliminated. Recourse was had to Livingston's "relative transpiration" method (19), but it was soon realised that this method was not adequate under all conditions, failing as it did to take into consideration the different effects of air movements on the rate of evaporation from different surfaces. Renner's work on various evaporating surfaces (26, *cf.* p. 128), showed that the effect of wind on the rate of evaporation from surfaces of different areas was not the same. Livingston (18) also showed that it was impossible to standardise different atmometers by comparison with a unit area of water surface or with any particular selected atmometer. It appears, therefore, that "relative transpiration" cannot under all conditions be considered to give a measure of the transpiring power of the plant.

The desirability of controlling the movements of air in the vicinity of the plant had early been recognised from *a priori* considerations, and efforts were made to obtain this control. After a series of trials with ordinary electric fans, a specially constructed

flue for the production of air currents of a constant velocity was designed by Professor Blackman and the writer. A description of this flue together with some account of its efficiency is appearing elsewhere (5). Having thus provided a means for controlling air movements in the region of the plant, or other evaporating surfaces, it was possible to test the value of the relative transpiration method. Experiments were conducted to determine the effect of variations of temperature, relative humidity of the air, and speed of movement of the air on the rates of evaporation of water from plant surfaces and from various atmometers. The results showed that relative transpiration is not necessarily a correct expression of the intrinsic transpiring power of a plant unless the velocity of the movement of the air is maintained constant (see Knight, 15).

The precautions and conditions which the foregoing work had shown to be necessary were adopted in planning and carrying out a further series of experiments in which records were obtained of temperature, of relative humidity, of rates of water-loss from plants and from atmometers of various types, of stomatal aperture and also of the water content of the plant under observation, as indicated by the difference between the amount of water absorbed and the amount lost by transpiration.

The experiments were conducted under various atmospheric conditions, involving both the normal daily fluctuations operating in a greenhouse, and the closely controlled environment which it is possible to maintain in a dark room. In all cases the air flue mentioned above was employed to ensure constancy of air movement. The writer concluded as the result of this work that both the stomata and the water content of the plant were important factors in the control of transpiration. It was found that the stomata did not, however, close in response to slight reductions of the water content of the leaf, but that they were far more sensitive to changes of illumination. On the other hand slight deficiencies in the water content were effective in reducing the rate of transpiration, and it was found possible to increase the rate of transpiration of a plant under controlled conditions by producing experimentally an increase in the water content of the cells. This work thus gave results which are in full agreement with those of Livingston and Brown and of Mrs. Shreve (29). Trelease and Livingston (30), using the porometer method, found that the stomata continued to open after the transpiring power of the plant had reached a maximum for the day, and from this and their earlier results they concluded that the reduction of the

transpiring power was due to the lack of water in the leaf and not to any stomatal control. Lloyd (24) found that the stomata were often opening when the water content of the plant was decreasing. In the experiments mentioned above, the present writer also frequently observed the same phenomenon, and incidentally, that the rate of transpiration was decreasing, *i.e.*, the reduction of transpiring power was due to the lack of water, which was more effective in altering the rate of transpiration than the opening of the stomata. At the same time it was observed that under suitable conditions it was possible for the transpiring power to increase while the stomata were closing, which was the result recorded by Trelease and Livingston.

The general inference from these results is that when the supply of water to the transpiring cells is sufficient to replace that lost by transpiration, the movements of the stomata may be of primary importance in the regulation of the rate of water-loss, as Darwin maintains, but when the rate of transpiration is high and the supply of water is insufficient to cope with the demand, then lack of water is a more important factor than stomata changes. The writer also agreed with Lloyd (24) that the stomata do not help to conserve the water of the plant by closure in response to slight reductions in the quantity of water in the cells.

The results outlined above are those which have been published up to the present, and it is proposed to give an indication of the lines along which the research has since progressed, and also of the methods and work which the writer contemplates when circumstances permit a return to the laboratory.

The direct influence of illumination changes on the rate of transpiration as indicated by Darwin's results (10) is one problem which has been attacked. Darwin found that when the influence of changing stomatal aperture is excluded, the rate of evaporation from the mesophyll cells is about 30% greater in daylight than in darkness, and that therefore this is a contributing factor to the well-known high diurnal transpiration rate of most plants. This problem was first attacked by the present writer early in 1914, and has also more recently been the subject of experiment. The rates of water-loss from slices of plant tissue (potato and turnip) in light and darkness were measured by weighing, the evaporating power of the air being kept fairly constant by means of drying agents and an electric fan. Water surfaces, wet filter-papers and films of gelatin were used as controls. The results showed that the rate of evaporation in the light was certainly not very con-

siderably greater than in the dark, but the limits of the method would not permit the conclusion that there was no difference at all, because although small and fairly consistent differences were obtained it seemed likely that they were within the margin of the experimental error. This method was accordingly abandoned, and more recently the air flue already described (5) has been used for experiments along the same lines. Cylinders cut from potatoes were suspended in the flue, and the rate of loss of water from them was determined by weighing, both under the ordinarily varying conditions of temperature and humidity in the laboratory, and also in the dark room where these factors were constant. In the former case various atmometers were used as controls. It has not been possible under these conditions to demonstrate any difference between the rate of water-loss from the tissue in the dark and the rate under the influence of a 1500 candle-power electric lamp placed two feet away. Time has not yet permitted a full test of the similarity or otherwise between the influence of this lamp and that of daylight on stomata and transpiration. A limited number of experiments on about six plants (mesophytes and xerophytes) showed that the effect of the artificial light on the stomata was very similar to that of diffuse daylight. Records of the stomatal behaviour of plants in the greenhouse were started, and after a time the plant and apparatus were removed to the dark room where they remained until the size of the apertures of the stomata had become constant. Then the electric lamp was switched on and it was observed that the stomata then opened to about the same extent as they did in the greenhouse on a dull day. Care was taken throughout to prevent the temperature of the air near the plant from rising unduly as the result of the heat generated by the lamp. To effect this a screen of running water from the tap was interposed between the lamp and the plant, and this was found to be a sufficient protection if the lamp was about two feet away.

No tests of the effect of the artificial light on the rate of transpiration have yet been made.

Experiments have also been carried out, in a manner similar to that just described for artificial light, on the effect of diffuse daylight on the rate of evaporation from potato tissues, and the indications are that there is no difference between the rates of water-loss in light and darkness.

It is proposed next to repeat, under more closely controlled conditions, the experiments described by Darwin. The original experiments were carried out in a constantly varying atmosphere

and apparently without precautions as to regulation or determination of the influence of the changing evaporating power of the air. By the use of atmometers and the air-flue, and by using Darwin's own method of eliminating the influence of stomatal change by blocking the pores with vaseline, and then slitting the leaves to provide a passage for the evaporated water to reach the outer air, it appears probable that a more exact idea may be obtained of the effect of light on the evaporating power of the mesophyll cells. Also artificial light can be used and experiments can be carried out in a dark room where temperature and humidity are practically constant, thus dispensing with the use of atmometers.

With the same apparatus and a slightly modified method it will be possible to investigate the effect of light on various tissues. The writer has in view a few plants accessible to him (which have been discovered by a lengthy process of trial and error) from the leaves of which the epidermis can be easily removed, leaving the mesophyll exposed. The effect of light on the rate of evaporation from the mesophyll tissue can thus be determined as in Darwin's experiments. Another possibility which may be of use if other results warrant its adoption, is the employment of the tissue of potatoes, the outer layers of which have developed chlorophyll as the result of prolonged exposure to light.

The same method offers the possibility of investigating the peculiar power of some cacti to transpire more by night than during the day (cf. p. 130 above). The application of the evaporation method along the lines indicated above should be of value in this connection by determining the resistance of the tissue to evaporation into the air instead of its power of absorption on immersion in water, which was the principle of Mrs. Shreve's work (28).

A considerable amount of work has been done on the mechanism of the movements of stomata, and a number of observations of stomatal behaviour have been recorded which have not yet been fully explained. The relation of the water content of the leaf to the size of the stomatal aperture has in recent years been discussed (see p. 130), and Iljin offered the outline of an explanation of the manner in which excessive transpiration might affect the stomatal aperture, but no evidence has been adduced in support of his theory that the enzymes of the cells may be actuated by changes in the water content. Lloyd (23) has also dealt with the relation of the size of the stomatal aperture to the contents of the guard cells.

In the course of the work on transpiration the present writer has collected a number of data with regard to the behaviour of stomata under various conditions, such as the loss of water from the leaf (see Laidlaw and Knight, 16), the effect of the continued exclusion of light from the plant and the existence of an induced rhythm of opening and closure. These details, however, at present lack correlation, and it is not proposed to publish them yet, but work has been intermittently in progress.

In 1916 a series of experiments was started to investigate further the phenomenon of the preliminary stomatal opening which occurs on reducing the water-supply of the leaf. The work involved the determination of the leaf water content at various stages of the process, but on account of the difficulties encountered owing to the variations of different leaves, the work promised to be laborious, and was therefore postponed in favour of other work then in hand until a more favourable opportunity should present itself.

It is hoped that by means of the self-recording apparatus mentioned above, such problems as these may be investigated, with the object of throwing more light on the question of stomatal change in general.

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ON THE FORCIBLE DISCHARGE OF SPORES OF *LEPTOSPHERIA ACUTA*.

BY WILLIAM J. HODGETTS.

[WITH 15 FIGURES IN THE TEXT].

THE following account of the mode of ejection of the ascospore, of *Leptosphaeria acuta* may prove of interest, especially since comparatively few Pyrenomycetes appear to have been examined critically for the purpose of finding the method of spore discharges

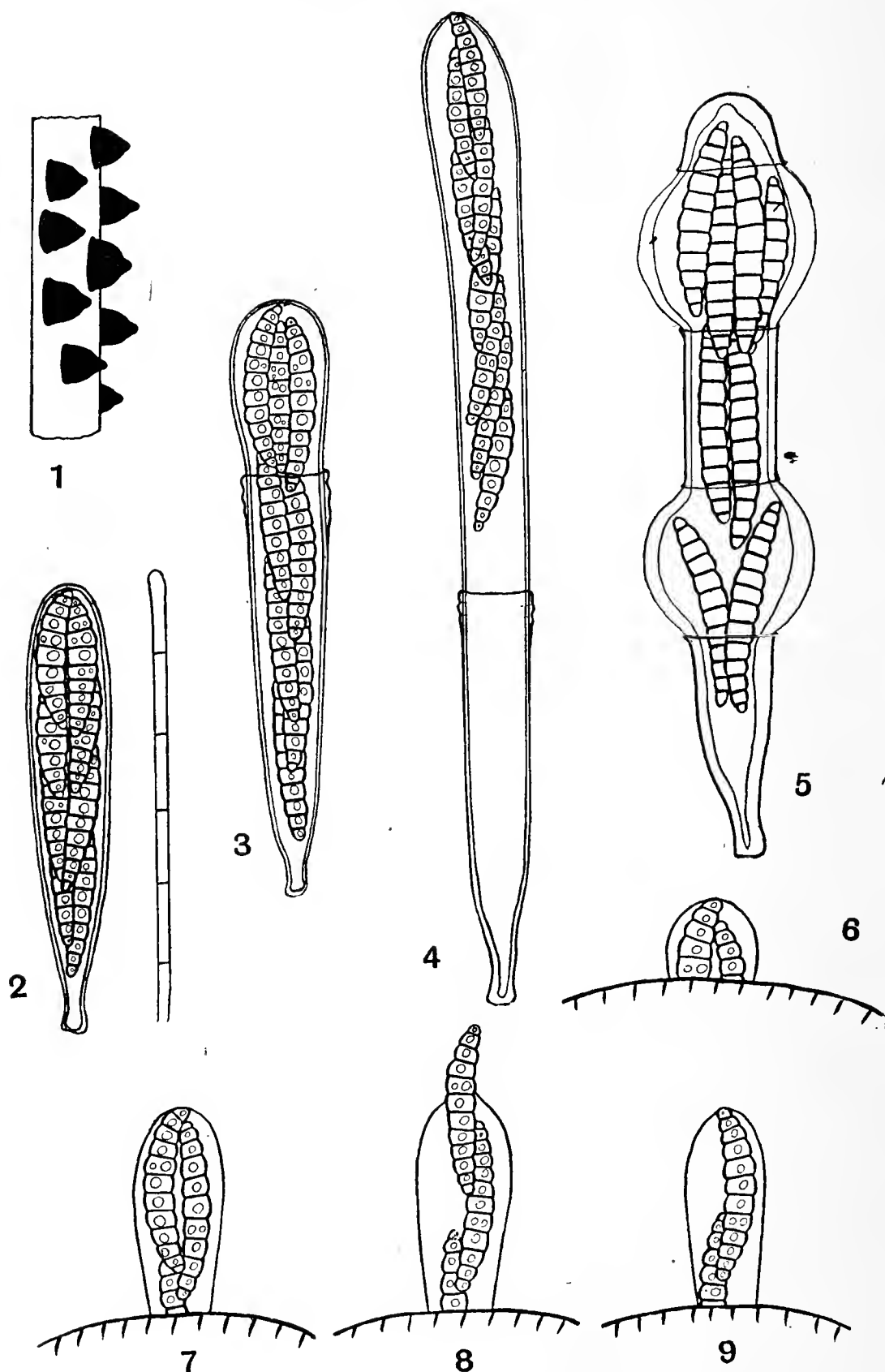
FIGS. 1-9. *Leptosphaeria acuta*.

FIG. 1. Group of perithecia in profile on dead nettle stem. FIG. 2. Mature ascus, with paraphysis on the right. FIG. 3. An ascus, the inner coat of which has just ruptured the outer at the apex. FIG. 4. The same a few seconds later showing the elongated inner coat. FIG. 5. An ascus that has dehisced irregularly owing to artificial pressure; the swollen inner coat is emerging through two annular splits in the outer coat. FIGS. 6 to 9. Successive stages in the ejection of the first spore through ostiole of a perithecium, observed in profile, in moist air.

FIG. 1, $\times 15$; FIGS. 2 to 4, $\times 300$; FIGS. 5 to 9, $\times 400$.

and since no account of the phenomenon in the present species seems to have been given by anyone previously.

Leptosphaeria acuta, a Pyrenomycete of the family Pleosporaceæ, occurs on herbaceous stems, and is very common over almost the whole of Europe. It is very readily to be found during winter and spring upon the bases of decaying nettle (*Urtica dioica*) stems. The black shining perithecia (Fig. 1) are separate, no stroma being present, and are at first immersed in the cortex of the stem, but by the decay and falling off of this cortex they come to be freely exposed, each being firmly fixed by its broad base to the wood of the stem. The perithecia are hemispherical or more or less conical, about $\frac{1}{3}$ mm. high, with a prominent wart-like ostiole; the wall of the perithecium is tough and coriaceous, and composed of dark-coloured plectenchyma. The asci (Fig. 2) are cylindrical when mature, 150μ to 200μ long, containing 8 fusiform biseriate yellowish-brown spores, each of the latter being 50μ to 58μ long and transversely septate. The number of septa varies, but usually there are from 10 to 13, while each cell contains one or several oil-guttules. The spores are often slightly curved. The hyaline wall of the mature ascus consists of two layers, an outer thin inextensible cuticular layer which does not swell when immersed in water, and an inner gelatinous coat which absorbs water very readily, and is thereby capable of swelling to a considerable extent. Among the asci are numerous filiform septate paraphyses whose walls are likewise somewhat gelatinous, and swell up in water.

When a ripe perithecium (the perithecia ripen during winter and early spring) is cut open and the hymenium carefully withdrawn with a needle and mounted in a drop of water on a slide, the rupture of the outer coat of the ascus wall by the inner coat at the apex of the ascus can frequently be observed, providing the asci are fully mature and have not been detached from the sub-hymenial layer, or injured in any way. This rupture (Fig. 3) of the outer cuticular coat at the apex is brought about by the increased turgidity of the ascus, induced by endosmosis, and probably also to some extent by the absorption of water and consequent swelling of the inner gelatinous layer. The latter breaks through almost explosively, and elongates *suddenly* through the apical cleft to about the distance shown in Fig. 3; that is, to about $1\frac{1}{3}$ times its original length, at the same time broadening out somewhat in its upper extruded part. The inner gelatinous tube with its spores, which always tend to remain in the upper part, continues to elongate, often at a fairly rapid rate, reaching in a few

seconds a length equal to three times the original intact ascus (Fig. 4), or even longer, but under these conditions was never observed to discharge its spores; it gradually dissolves and the spores germinate *in situ*. During this process of elongation the inner coat of the ascus wall can be seen to be quite free from the outer cuticular coat in the neighbourhood of the upper part of the latter at any rate; lower down the two coats are firmly joined. The free upper part of the outer membrane becomes slightly retracted, and usually shows a few transverse folds or wrinkles. When the perithecia are simply crushed under a cover-glass and the asci expelled, the outer coat of the ascus wall is frequently observed to have been ruptured by one or more annular splits as shown in Fig. 5, the inner coat swelling and emerging in various irregular ways.

If a few mature perithecia are mounted whole in water, and observed in profile, after an hour or so the successive emergence through the ostiole of the inner gelatinous coats of the asci, each containing eight spores in its upper part, can readily be observed. Each elongates, sometimes at a very rapid rate, to about 0.3 mm. or even more beyond the orifice of the perithecium; its wall can be seen to be thin and tightly stretched. However, under these conditions the spores were never seen to be forcibly ejected; the gelatinous tubes after elongating to a considerable length appear to break away at the base, since each is usually observed to suddenly collapse, while the spores being relieved from the pressure which kept them in the apical region of the tube suddenly shoot *down* the latter and come to lie scattered lower down the ascus tube, although still outside the perithecium. The numerous collapsed asci with their spores eventually form a tuft radiating from the ostiole of each perithecium, and finally, by dissolution of the walls of the tubes the spores are set free in the water. This mode of spore dispersal probably takes place in nature when the perithecia are wetted with rain or dew.

So far we have spoken of the behaviour of the asci when immersed in water, and have observed how the ascus dehisces and the inner wall with the spores elongates, but if perithecia are kept in a moist atmosphere, that is, under somewhat more normal conditions, then a very different process takes place. When pieces of nettle stems with numerous mature perithecia attached are placed in a Petri dish, the bottom of which is covered with damp blotting paper, and left for 24 hours, the under side of the lid of the dish, several millimetres above the upwardly directed ostioles, will be found to have numerous spores adherent to it, thus showing

that the spores have been forcibly ejected from the asci. In order to observe the process under the microscope, mature perithecia were carefully removed with the point of a scalpel (it is an advantage to remove a small piece of the wood of the stem below each perithecium at the same time, to ensure the base of the latter being uninjured) and affixed laterally to a cover-glass by means of a very minute drop of gum solution; the cover then being inverted over a drop of water contained in a deep cell cemented on a slide, the edges being sealed with vaseline. The perithecia can now be observed in profile under a fairly high power, and if the observer has sufficient patience the actual discharge of the spores through the ostiole will sooner or later be seen. The process is as follows.

The tip of the gelatinous inner wall of an ascus appears through the ostiole (Fig. 6) and continues to elongate, sometimes slowly, but at other times rapidly, occasionally very rapidly, to a distance equal to 1 to $1\frac{1}{2}$ spore-lengths above the aperture (Fig. 7). Usually 2 or 3 spores lying side are visible within the tube, the tip of the uppermost spore being pressed firmly against the extreme apex of the tube, the wall of which is here thinner than elsewhere, and, after the tube has elongated to the distance mentioned, bursts through the apex (Fig. 8), the spore being shot out through the pore with the speed of an arrow. Immediately the first spore has been discharged the tube suddenly contracts a short distance (very approximately equal to 5μ), due, of course, to the pressure within it being slightly reduced by the ejection of the spore; however, simultaneous with this short contraction the second spore is forced into and stops up the pore (Fig. 9) while the ascus elongates to about its original length. The second spore is forcibly shot out like the first, and the third spore pushed up into the pore immediately afterwards, and so on until all the 8 spores have been ejected. After the discharge of each spore the tube always contracts a little, and the next spore is forced so rapidly into the place previously occupied by the spore just discharged, that the one seems to replace the other without any appreciable interval of time. After this contraction the ascus always elongates again nearly if not quite to its former length before the next spore is ejected. The spores are discharged in rapid succession, the time required for the discharge of the 8 spores of an ascus from the time the first spore is ejected to the shrinking of the empty gelatinous tube back through the ostiole, being usually only 4 or 5 seconds. Sometimes the process is interrupted, 3 or 4 spores being discharged, and the tube then withdrawing into the perithecium to elongate and discharge the remainder later. The reason why the spores never

seem to be ejected when the asci are observed in water appears to be due to the too great elongation of the inner ascus tube under these conditions, the internal pressure thus not rising to the extent required forcibly to eject the spores. In moist air the ascus tubes never emerge through the ostiole as far as they do when the perithecia are in water.

In a damp atmosphere it was found that the spores could be thrown a horizontal distance of 0.4 to 2.0 cms. (usually about 1 cm.); and could be shot vertically to a height of 0.2 to 1.2 cms. above the ostiole. These distances are greatly exceeded by certain other Ascomycetes in which explosive discharge of the spores is known. De Bary¹ states that in the strongly puffing fungi, such as *Peziza vesiculosa*, *P. acetabulum*, *Helvella crispa* and *Ascobolus furfuracens*, the spores are thrown to a distance of more than 7 cms.; in *Sordaria fimiseda* they may travel 15 cms., and in the smaller species of this genus about 2 cms.; in *Rhytisma acerinum* only a few mms. According to Buller² the spores of *Peziza repanda* are shot vertically a distance of 2 to 3 cms. above the hymenium, while those of *Ascobolus immersus*³ are discharged vertically to a height of 25 cms., or even more, the maximum being 35 cms., while they could be shot horizontally 30 cms. The last example exhibits the greatest violence of spore-discharge known in any Ascomycete (although it is easily beaten by *Pilobolus*). These species discharge all the spores of the ascus simultaneously, the method commonly found in Discomycetes, but occurring also in some Pyrenomycetes (e.g., *Sordaria*), and in *Protomyces*.

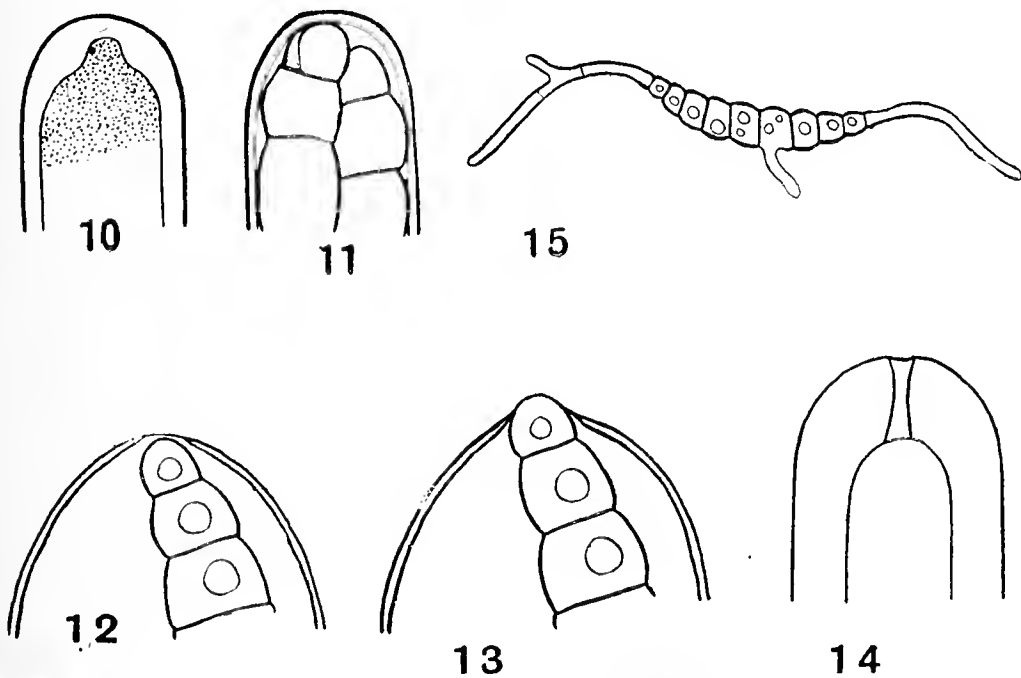
Certain characteristic changes in the apical region of the ascus wall of *Leptosphaeria acuta* can be traced during the maturation of the spores. The young ascus, filled only with granular protoplasm and oil-drops, shows at the extreme apex a circumscribed thinner part of the inner coat of its wall (see Fig. 10), around which the latter is much thicker than elsewhere. The outer cuticular membrane can just be discerned, and appears to be uniformly thin over the apex of the ascus. When the spores are mature but the outer coat still intact the ascus is very turgid, and the inner coat appears thinner and uniform at the apex (Fig. 11), due no doubt to the fact that it is tightly compressed between the unyielding outer membrane and the turgid contents of the ascus. The two coats are now usually discernable only with difficulty (Fig. 11). Exactly what causes the inner coat to break through

¹ "Morph. and Biol. of the Fungi" Eng. Ed., 1887, p. 92,

"Researches on Fungi." 1909, p. 236.

Buller," *loc. cit.*, p. 252.

the outer at the apex is not quite clear; probably the outer firmer layer is somewhat thinner or weaker at this point. When the inner layer has broken through and commenced to elongate its extreme tip can be seen to be much thinner than the wall of the tube elsewhere (Fig. 12) which is of very uniform thickness. Doubtless this thinner apical part, through which the first spore is forced, is foreshadowed by the thin apical region of the inner coat seen in the young ascus. The thickened upper part of the inner coat also seen at this young stage seems to be a reserve piece of thickening destined ultimately to be extended when the tube elongates, as suggested by de Bary, although the whole inner wall is capable of stretching to a considerable extent. After discharging its spores the gelatinous tube collapses and shrinks back considerably, and, if in water, its wall becomes greatly swollen (Fig. 14).



FIGS. 10 to 14. *Leptosphaeria acuta*.

FIG. 10. Tip of very young ascus. FIG. 11. Tip of mature ascus. FIG. 12. Apex of elongated inner ascus wall before first spore has been ejected, showing the thinner apical part of wall. FIG. 13. The same with the first spore breaking through the apex. FIG. 14. Tip of empty and collapsed inner ascus wall showing the apical pore and greatly swollen wall. FIG. 15. Spore germinating in moist air.

FIGS. 10, 11 and 14, $\times 500$; FIGS. 12 and 13, $\times 1200$; FIGS. 15, $\times 400$.

The account given above for *Leptosphaeria acuta* is exactly the same as described by Pringsheim¹ for *Sphaeria Scirpi* (= *Pleospora scirpicola*), which occurs on decaying submerged parts of stems of *Scirpus lacustris*. Here the inner gelatinous coat of the ripe ascus suddenly breaks through the apex of the outer cuticular layer and elongates in a few seconds to about three times the length of the original ascus; the spores being shot out success-

¹ Pringsheim, Jahrb. wiss. Bot. Bd. 1, 1858, p. 189; or see de Bary, loc. cit, p. 93.

ively through an apical pore. *Sphaeria Scirpi*, however, apparently differs from *Leptosphaeria acuta* in being able forcibly to discharge its spores while the perithecia are under water. De Bary also mentions *Sphaeria* (*Leptosphaeria*) *Lemaneæ*, *Phyllachora Ulmi*, *Cordyceps* and *Claviceps*, as examples in which the spores are successively discharged in a forcible manner. Doubtless many other Pyrenomycetes discharge their spores in this way—especially among the Pleosporaceæ where the ascus wall has the characteristic double structure described above in *Leptosphaeria*.¹ However, it does not follow that all those forms with a double ascus wall discharge their spores explosively, although in all the Pleosporaceæ the inner gelatinous layer breaks through the apex of the outer membrane and elongates considerably, the spores following this elongation *pari passu* in the upper part of the tube. This suffices to carry the spores out through the ostiole, where, providing they are not forcibly ejected, they may be washed away by rain or dew, the process being the same as that occurring in *Leptosphaeria acuta* when the perithecia are in water. In the common *Pleospora herbarum* de Bary² mentions that the spores usually remain in the elongated ascus tube, although sometimes they may be shot out forcibly. Thus both methods of spore-dispersal may occur in one and the same species, as is the case with *Pleospora herbarum* and *Leptosphaeria acuta*, as well as probably many others.

The spores of *Leptosphaeria acuta* readily germinate in moist air a few hours after their discharge, the two end cells of each spore usually putting out germ-tubes first (Fig. 15), followed later by others from one or two of the central cells. No case was observed where every cell of a spore had sent out germ tubes.

Some simple experiments were conducted in order to determine whether light has any directive influence upon the discharge of the spores, but no such influence could be detected. The spores were found to be ejected in the same direction when the perithecia were subjected to strong unilateral illumination, as they were when in darkness, or illuminated equally all round. Heliotropic sensibility, such as is shown by the asci of *Ascobolus*, would not be of much use in the case of the present species, the perithecia of which are clustered on vertically placed dead nettle stems, and point their ostioles horizontally to all points of the compass, thus being very unequally illuminated. The spores are presumably ejected on to and infect the young nettle shoots which come up in spring, the time when the asci are mature.

¹ See Engler and Prantl, "Nat. Pfl.," Teil I., Abt. I. 1897, p. 428.

² *Loc cit.*, Legend of Fig. 47, p. 95.

NOTE ON THE DISTRIBUTION OF SEXES IN
MYRICA GALE.

BY A. J. DAVEY, M.Sc., AND C. M. GIBSON, B.Sc.

[WITH PLATE I AND ONE FIGURE IN THE TEXT.]

MYRICA GALE, the common Bog Myrtle, is described as typically dioecious, thus agreeing with the majority of the other members of Myricaceæ. The present note calls attention to the characters of various types of monoecious individuals and to variations in sex.

The occasional occurrence of some expression of the monoecious condition has been noted by several authors.¹ Thus Chevalier² mentions the exceptional presence of monoecious shoots, and of androgynous catkins, and Marshall Ward³ states that hermaphrodite flowers may occur.

Observations during several successive years on a large area of *Myrica Gale* in the peat moors of Somerset show that there always exists a small proportion of monoecious plants which present all gradations between the normal staminate and pistillate types. The series includes plants bearing catkins which contain hermaphrodite flowers. This has been found to be true as regards other areas in different parts of the country.

Further it has been found that the sex (if it may be so termed) of a bush or shoot may vary from year to year. The variations observed during several years have been almost entirely in the direction of change from the pistillate to the staminate condition; but in the present season (1916) several instances of the reverse change have been noted.

The Somerset area includes situations differing considerably in level, and consequently differing as regards moisture conditions. The low levels produced by the removal of a considerable depth of peat are always wet and boggy, and are flooded to various depths during the winter; the high levels consist of dry peat which is never flooded.

In all situations, staminate plants appear to be more numerous, but the relative proportion of pistillate plants was found to be greater in the wet than in the dry areas. Thus in a dry area

¹ Moss, C. E. The Cambridge British Flora. Vol. II., Camb. Univ. Press, 1914, p. 69.

² Chevalier, Aug. Monographie des Myricacees. Mem. Soc. Sci. Nat. Cherbourg, XXXII., 1901-2, p. 178.

³ Marshall Ward, H. "Trees." Vol. III., Camb. Univ. Press, 1905, p. 204.

the proportions were roughly:—staminate 75%, pistillate 10%, the remaining 15% being monœcious or hermaphrodite. At the same time, in a very wet area, the proportions were:—staminate 56%, pistillate 38%, hermaphrodite or mixed 6%. This represents countings of shoots traceable to the ground, which cannot with certainty be taken to represent individual plants. Since the staminate plants are always much more vigorous than the pistillate it is probable that the excess of staminate over pistillate individuals may be more apparent than real, although in the dry levels large areas of plants are met with, in which the pistillate type is almost entirely lacking.

As is well known, the catkins are borne towards the ends of special shoots, and up to the time of flowering the staminate catkins are much larger than the pistillate. The bracts of the staminate catkin are broad and spreading, while those of the pistillate catkin are narrow and compactly arranged. In both male and female catkins, the flowers occur singly in the axils of the bracts. The male consists of a group of four (sometimes more) stamens, without scales or bracteoles; the female consists of a bicarpellary ovary flanked by two adnate scales or bracteoles, which enlarge considerably after fertilisation to form the characteristic wings of the fruit. As the pistillate catkin develops its axis lengthens and becomes woody, while its bracts enlarge and become green. The woody axis persists long after the fruit has been shed, thus providing an indication of the amount of fruit that has been set in previous seasons.

The monœcious or "mixed" plants may be grouped as follows, but all the types grade into one another.

A. Plants or shoots bearing staminate and pistillate catkins of the normal type. These may occur on different shoots, or both may be found in the same shoots. Staminate, pistillate and mixed shoots may be found on the same plant. On the mixed shoots staminate catkins are generally found below, and pistillate above, but the reverse order may obtain. Androgynous catkins may be found in the intermediate region. There is much variation in the proportions of the different kinds of shoots present.

B. Plants bearing androgynous catkins. These catkins are large and resemble the normal staminate catkins as regards form, and the size and shape of their bracts. Stamens generally occur in the lower part of the catkin and pistils above; this is the arrangement commonly met with in those species of *Myrica*

in which androgynous catkins are the rule (*e.g.* *M. conifera*, described by Chevalier¹). Sometimes the reverse arrangement is found. The middle regions of these catkins may contain hermaphrodite flowers or flowers with rudimentary pistils.

C. Plants with shoots the bulk of whose catkins consist of hermaphrodite flowers. The catkins with hermaphrodite flowers are intermediate in size between the normal pistillate and staminate catkins, but may incline towards either kind in form and appearance, according to the nature of the bracts. The larger staminate-like type with broad and wide-spreading bracts is the more common. The pistillate type is smaller and more compact. The hermaphrodite flower contains a centrally placed ovary, similar to that of the normal female flower. Round it there are three or four stamens united to the base of the ovary, and just below these on the very short floral axis are two minute lateral outgrowths, corresponding to the "bracteoles" of the normal female flower. (See Fig. 1.) Both stamen and stigmas are functional, and the flowers are proterogynous. They are capable of producing fruit.

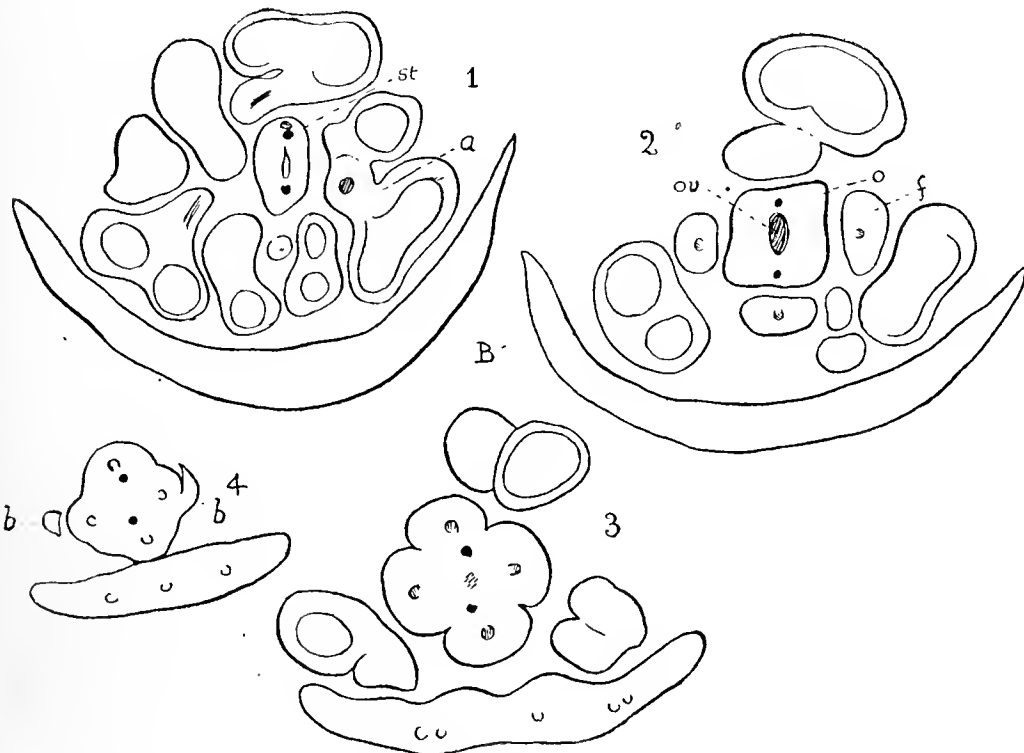


FIG. 1.—Transverse sections of hermaphrodite flower at different levels, showing ovary and four stamens (1, 2, 3, 4, numbered downwards). *St*, style; *o*, ovary; *ov*, ovule; *f*, filament; *a*, anther; *b*, bracteole; *B*, bract. Vascular bundles supplying styles are shown black.

In the upper scales of the catkins various abnormal stages may occur; *e.g.*, a centrally placed pair of stamens, flanked by two

¹ Chevalier. *loc. cit.*, p. 229.

small green scales corresponding in position to the "bracteoles" of the normal female flower, was found persisting, while fruits were developing in lower parts of the catkin. It is common to find rudimentary ovaries in which the "bracteoles" are replaced by a pair of stamens.

Variations in Sex from year to year.

Bushes and shoots of various types have been marked and examined in successive years, their previous state as regards fruit production being recorded. There is considerable difficulty in identifying an individual since the plant possesses underground stems. The changes recorded include only such as took place on the branches of single shoots traceable to the ground; in a few cases where a tree or a bush is mentioned, reference is made to isolated tree-like plants possessing only one stem or trunk.

In fourteen cases clumps or bushes found to be entirely pistillate in 1913 and in 1914, produced staminate catkins in 1915, in some cases abundantly, and in others sparsely. One plant became almost entirely staminate. In some cases staminate shoots were produced, the upper catkins of which were hermaphrodite; other branches bore pistillate and staminate shoots in about equal numbers, while a few remained for the most part pistillate, producing only a few staminate shoots. Thus sometimes the change may be generally spread over a tree or clump of bushes, while in others only certain shoots may be affected. In the latter case there may be progressive change extending over several seasons, since in successive years the proportion of staminate catkins increased. Thus certain trees and branches which produced abundant fruit in 1913 developed mixed shoots in 1914, and in 1915 had become almost entirely staminate. In 1916, however, some of these showed an increase in the production of pistillate catkins.

It would seem that it should be possible to gain some insight into the causes underlying these variations in sex, and to ascertain whether external conditions play any part in determining the proportions of the sexes which appear. The conditions which naturally suggest themselves are moisture, temperature, and light (with their influence on nutrition), and also the previous state of a plant as regards the production of fruit.

Since the staminate flowers are developed early in the season preceding that in which they flower, while the pistillate catkins

develop much later, it is possible that conditions accelerating or retarding the development of catkin buds may influence the proportions of the two kinds.

Variations similar to those above recorded for *Myrica Gale* are described by Chevalier as occurring to a marked extent for certain monœcious species of *Myrica* (notably *M. californica*, *M. conifera* and *M. pubescens*), in which a polygamous condition obtains. Chevalier suggests nutrition as the factor which controls the distribution of the sexes on monœcious individuals in any one season, as well as the changes taking place in different seasons.

In conclusion, our thanks are due to Dr. E. N. Thomas for encouragement and helpful criticism.

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DESCRIPTION OF PHOTOGRAPHS ON PLATE I.

1. Different kinds of flowering shoots (April, 1913), *a*, *b*, *c*, *d*, reading from left to right.
 - a*. Branch with staminate catkins, showing remains of small amount of fruit of previous years.
 - b*. Branch with remains of fruiting catkins (1912) bearing one staminate and two pistillate shoots.
 - c*. Branch terminated by fruiting shoot of 1912, below which have arisen shoots bearing staminate and "hermaphrodite" catkins.
 - d*. Is entirely pistillate, with remains of previous season's fruiting catkins.
2. Fruiting, branch in August, bearing a shoot with young staminate catkins for next season.
3. Branch bearing staminate catkins on 1915 shoots. On the left is a bare persistent staminate shoot of the previous season, and on the right a fruiting shoot showing woody axes of catkins and bracts persisting after the fruit has been shed.

RADICAL LEAVES OF *PARNASSIA PALUSTRIS* AND *VALERIANA DIOICA*.

BY H. STUART THOMPSON, F.L.S.

[WITH TWO FIGURES IN THE TEXT].

CONFUSION may possibly occur between young plants of these two species (which often grow in the same habitat) owing to the superficial similarity of their radical leaves. The writer fell into this mistake two years ago, believing he had gathered the former plant, which had not been recorded from Somerset since

1782, in a bog below Blackdown, Mendip. In 1903, also, he mounted a fine seedling of *V. dioica* with flowers of *P. palustris* from Askham Bog, Yorks, where both plants grow.

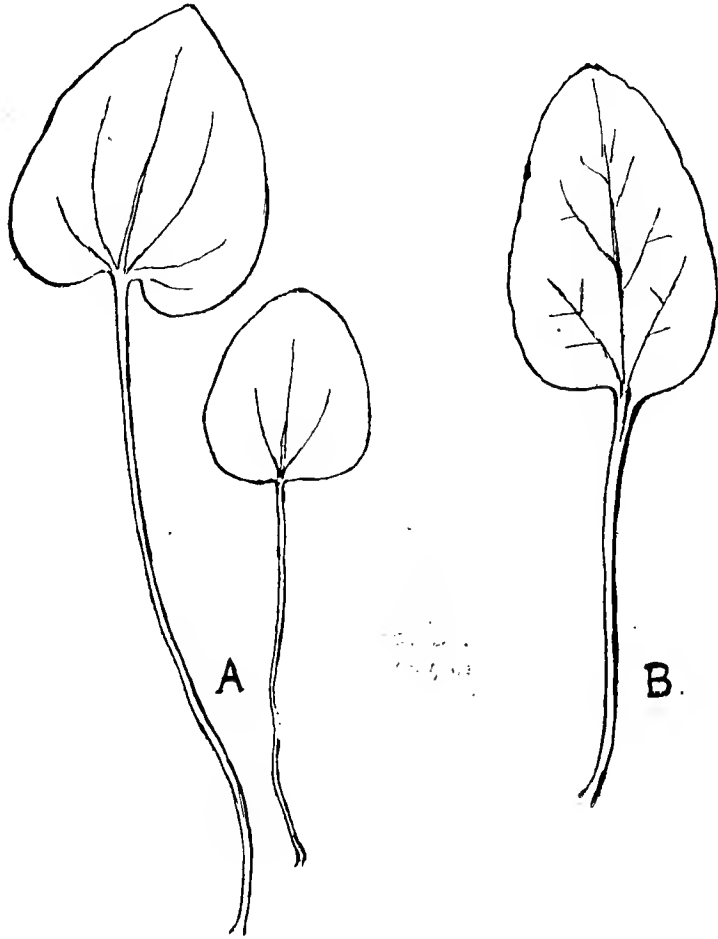


FIG. 1. A. Radical leaves of *Parnassia palustris*. B. Radical leaf of *Valeriana dioica*. Drawn from herbarium specimens.

The general similarity of the radical leaves of the two plants is shown in Fig. 1, which also exhibits one of the leading differences, *viz.*, that *Parnassia* has palmate and *Valeriana dioica* pinnate venation. On careful examination further differences become apparent. The leaves of *Parnassia* are more leathery and have more slender petioles than is the case in the marsh valerian. The radical leaves of *Valeriana dioica* are also ciliate,¹ being edged with short stiff white "cilia" which sometimes continue down the petiole. The veins on the under sides of the leaves are also densely pitted with minute white pores. Both the "cilia" and the pores are hydathodes encrusted with calcium carbonate. The yellowish-brown spots, due to tannin cells, on the lower surfaces of the leaves of *Parnassia*

¹ None of the British floras in common use, except Hooker's "Student's Flora," mentions the ciliation of the leaves of *Valeriana dioica*.

constitute another point of difference (Fig. 2). These are mentioned by Mrs. Arber,¹ who concludes that their presence gives little support to the supposed Saxifragaceous affinity of *Parnassia* and that the relationship of this genus with Hypericaceæ is closer than was supposed by Drude. Mrs. Arber accepts Drude's view that the genus should be placed in a separate family—Parnassiaceæ.

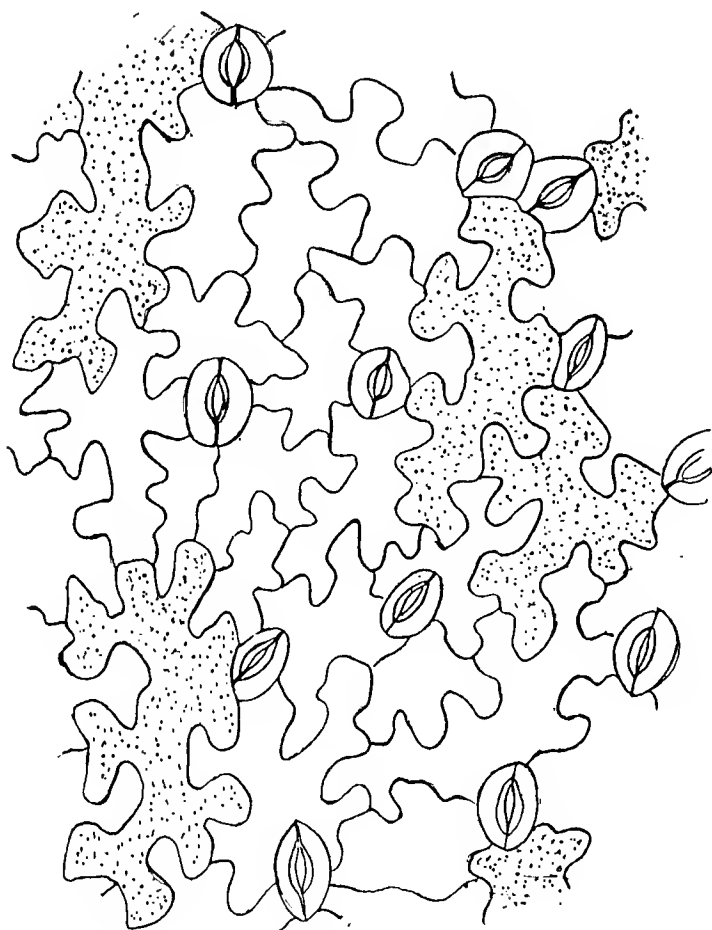


FIG. 2. *Parnassia palustris*. Epidermis of lower surface of leaf showing tannin cells (dotted). \times about 200. From an unpublished drawing by Mrs. Arber.

¹ Agnes Arber, "On the structure of the andrœcium in *Parnassia* and its bearing on the affinities of the genus." *Ann. of Bot.* 27, July, 1913, p. 502. Mrs. Arber has kindly placed at my disposal an unpublished drawing (Fig. 2) showing the distribution of these tannin cells on the underside of the leaf. Cf. Mrs. Arber's paper for references to the original description of these cells.

Since this note was in type, I am reminded that the late Mr. Townsend, in his "Flora of Hampshire," asks "Can it [*Parnassia*] have disappeared [from the New Forest], or did Mr. Mill [John Stuart Mill] visit the forest before it was in flower and mistake the leaves of *Valeriana dioica* for those of *P. palustris*? I know an instance of such a mistake being made by an acute botanist." [H. S. T.]

RUTH HOLDEN (1890–1917).

IN previous numbers of the *NEW PHYTOLOGIST* some account has been given of the lives and work of British Botanists who have fallen on the battlefields of France, and it is now my sad privilege to pay a tribute to the memory of an American citizen and Botanist, Ruth Holden, who fell a victim to disease while serving with a British Medical unit in Russia. Ruth Holden was born at Attleborough, Massachusetts, in 1890 and was the youngest child of Dr. John Holden; she was educated at Attleborough High School and later at Radcliffe College, the Women's Department of Harvard University. She graduated B.A. in 1910 and in the following year became a Master of Arts of the University.¹ On the completion of her student's course, Miss Holden devoted herself more especially to the anatomical study of recent and fossil Conifers and was a loyal adherent to the views of her teacher, Professor Jeffrey. In 1912 she paid her first visit to England to attend a "Summer Meeting" at Cambridge and to examine collections of fossil plants. Soon after her return to America, Professor Jeffrey wrote: "Miss Holden has returned and has taken a great fancy to Cambridge of the Motherland. She is going to make every effort to make a year of it with you, next year or possibly a little later. I am glad you like her; she is certainly exceptional among the young women with whom I have come in contact." In 1913 she returned to this country as a Harvard Travelling Fellow and began work at Cambridge, attending lectures and pursuing palaeobotanical research. Her training had been rather intensive than extensive and one of her aims was to obtain a good grasp of Botany in the wider sense while taking every advantage of the opportunities of research afforded by British collections and fossiliferous localities. She made collections of petrified Jurassic wood on the Yorkshire coast and on the coast of Sutherland, and many of the specimens she insisted on cutting herself with an old machine which had long been neglected in the basement of the Botany School. A collection of Indian plants, both Palaeozoic and Mesozoic, lent to me by the Director of the Indian Geological Survey, supplied much interesting material which she attacked with her usual thoroughness and skill: her method was to make a preliminary study of several recent forms and in dealing with the fossils she spared no pains or ingenuity in extracting the last ounce of evidence from the indifferently preserved

¹ I am indebted to Miss Jordan Lloyd, Fellow of Newnham College, for several of the facts mentioned in this article. A.C.S.

specimens. Our intention was to publish a joint description of the Indian collections. Miss Holden was an excellent critic and, while firmly convinced of the correctness of views which I ventured to call in question, she was always able to give reasons for the faith which was in her.

The greater part of her work was concerned with the affinities of Cretaceous and Jurassic Coniferous stems and to this difficult branch of investigation she made several important contributions; she also added considerably to our more precise knowledge of the nature of some Indian fossil Conifers and certain Cycadophytan European genera. In the summer of 1911 she collected material in Prince Edward Island and on the south shore of New Brunswick which led to the publication of a paper in the *Annals of Botany* in 1913. Shortly before leaving England she completed an account of a new type of petrified stem of Cordaitalean affinity from the Lower Gondwana rocks of India which has not yet been published. While recognising the value of the work accomplished in the short time that had elapsed since the completion of her degree course, those who had the privilege of knowing Miss Holden were convinced that she would accomplish much more in the future; she had the qualifications which make for success, a strong personality, modesty combined with wholesome self-confidence, untiring energy, and enthusiasm.

Miss Holden became a post-graduate student of Newnham College and was afterwards elected to a Fellowship, an event which gave her genuine satisfaction. Soon after the outbreak of the war she attended the first course of Red Cross lectures given at Cambridge and served in a hospital for convalescent Belgian soldiers. Anxious to obtain a more thorough training she spent three strenuous months at Addenbrooke's Hospital with a view to a post in a British Military hospital, but to her great disappointment her nationality proved to be an obstacle. After an unsuccessful attempt to return to scientific work she succeeded in the face of many difficulties in being admitted a member of the first Millicent Fawcett Medical Unit equipped in this country for service in Russia. In December, 1916 she temporarily resigned her Fellowship at Newnham and left for Petrograd. Miss Jordan Lloyd writes: "With the whole-hearted enthusiasm with which she carried out any work she undertook, she set to work to learn Russian and so successful was she that ultimately she was appointed interpreter and courier to the Unit. She worked for a time in Petrograd and then

went ahead with a pioneer party to prepare and equip a new hospital for Polish refugee children at Kazan. Here she found herself with a little precious spare time on her hands, and as the University classes were in full swing she introduced herself to the Botanical Staff and set to work on some fossil plants in the Museum in which she was especially interested. She wrote at this time that she hoped later to return to Kazan to complete her examination of the material. The Unit again claimed her whole attention and she worked with it for a time at Kazan, travelling later to another Unit stationed in Galicia." She spent much time in travelling between Petrograd, Galicia and various parts of Russia, distributing stores among different hospitals. In January news was received that she was lying ill of typhoid fever at Kazan; later information led her friends to believe that she had completely recovered, but meningitis supervened, and she died at Moscow on April 21st. Miss Moberley, the Administrator of the Unit, writes: "She journeyed the length and breadth of Russia by herself, picking up the language and acting as messenger, accountant, storekeeper, V.A.D., or whatever else was required of her, with a zest which viewed even acute discomfort and hunger as a 'lark,' while she showed a cheery patience and self-effacement under the most trying circumstances which won my heartfelt admiration." To quote again from her close friend, Miss Jordan Lloyd: "Her keen clear mind, her geniality, her never-failing courage and good humour made her friends wherever she went. To her friends her death is an irreparable loss. Her life, though short, was crowded to the full with excitements and adventures which she loved, and the crowning adventure of self-sacrifice is one from which she herself would not have shrunk. She came to the help of the Allies while her country was still a neutral power, feeling it incumbent on individual Americans to do all they could by private endeavour. The entry of the American nation into the war, which she just lived to see, must have filled her cup of happiness to the brim."

In endeavouring to express a keenly felt sense of loss occasioned by the untimely death of a student of Nature, it sometimes happens that the human qualities gradually but firmly exercise a preponderating influence upon the mind of the writer: though Miss Holden was a student of exceptional originality and promise she was much more than that—a chivalrous and noble woman whom it was a privilege to count a friend.

A. C. SEWARD.

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THE ORIGIN AND DEVELOPMENT OF THE COMPOSITÆ.

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INTRODUCTION.

THE present essay is an attempt to throw light on some of the problems of evolution by the intensive study of a single successful group. In 1874, when the section on Compositæ of Bentham and Hooker's *Genera Plantarum* (8) was published, the family included over 10,000 species; in 1897 when Hoffman completed the corresponding section of Engler and Prantl's *Pflanzenfamilien* (42) the number had risen to over 13,000; in the *Index Kewensis* and its supplements up to 1910 there are approximately 30,000 species in the family. In such a well-defined and closely inter-related family the genera tend to be founded on slighter differences than in other families and the species tend to become elementary or completely artificial rather than Linnean, so slight are the differences which are here considered to be sufficient for taxonomic purposes. In spite of this technicality the larger groups are more or less natural and the numerous intermediate forms between genera and tribes make the family a favourable subject for evolutionary studies.

The general habit varies so much with climatic and geographical situation that the vegetative characters are of little value in the study of the inter-relationships of the tribes, but there are cases where these features, added to well-marked floral relationships, confirm the lines of development already indicated. The physiology (irritability of the pollen-presentation mechanism, latex, etc.) and the cytology can be shown to develop in conjunction with the changes in floral structure, but ultimately the morphology of the flower and capitulum is the real test of relationship and development. The study of the details of the flower in this homogeneous group

becomes, therefore, particularly important, and as there is least variation in the essential parts of the flower these must be considered more important than the very variable non-essential parts. This was recognised by the first synantherologist, Cassini, and is confirmed by Bentham, the only other botanist who has studied the *Compositæ* sufficiently profoundly to express a sound and independent opinion.

After dealing with the history of the *Compositæ* we shall, therefore, consider the variation in the form and structure of the styles and stamens. As these are closely connected in the work of pollen-presentation the development of that mechanism, including its susceptibility to stimuli, is of first importance.

The variations in the corolla are considered in relation to the floral development and insect visitors; the variations in the pappus are considered in relation to the component parts of the trichome and the dispersal of the seeds. The characters of the receptacle and involucre are considered in relation to their development as indicated by closely allied forms of receptacle and involucre and in relation to the lines of evolution suggested by the examination of less variable parts of the capitulum. The various phylogenetic lines indicated by the critical examination of these floral characters are tested by the geographical distribution of the groups and the phyllotaxis and cytology of the family are found to follow the phylogenetic lines established by the preceding investigation.

In the discussion of the inter-relationships of the tribes and genera, use is made of the modern theories of heredity, evolution and geographical distribution. Some of these may be regarded as rather speculative, but their use is considered justified by the coherent account of the evolution of the family which results. Lines for further research are abundantly indicated and correction or confirmation of the views expressed will be sought along the various lines of physiological relationships, cytological and geological development and the details of geographical distribution.

CHAPTER I.

HISTORY OF THE GLASSIFICATION OF THE COMPOSITÆ.

In the history of synantherology the classifications of the taxonomists have been in the majority of cases more or less artificial attempts at grouping related genera and only a few have made any attempt at expressing the affinities of the tribes and sub-tribes. On the other hand a number of students of the Compositæ have expressed views concerning the relationships of the tribes, but these views have been founded in some cases on somewhat limited enquiries into the anatomy and morphology of a number of genera.

It has been found convenient, therefore, to discuss the history of the grouping of the genera into tribes and the history of the grouping of the tribes according to their supposed relationships in separate sub-sections.

A. TAXONOMY.

The present sub-section deals not so much with the precise details of the classification of the family as with the evolution of the idea of the family as such and of the ideas of the different tribes as groups. It is an attempt to trace this evolution from the first more or less sub-conscious grouping of *Sonchus*, *Cichorium* and other Cichoriæ by Theophrastus to the modern conceptions of the divisions and sub-divisions of this immense and homogeneous mass of species.

Ancient Systems.

Theophrastus. *Circa* B.C. 320.

The origin of the idea of two of the tribes of the family, the Cichoriæ and Cynareæ, seems to be lost in antiquity like the origin of some other very natural groups, which, as Greene (37) says, must have been recognised by primitive man from the earliest times. They are distinguished very clearly by Theophrastus in his *Enquiry into Plants* (83). The chicory-like plants (χίχωριώδης) discussed in Book VII, Chapter XI, include *Cichorium*, *Hypochaeris*, *Taraxacum* and *Picris*, all described and distinguished from one another and given, moreover, only as examples of the class. The distinguishing characters of the group are given clearly and concisely. Theophrastus also mentions in Book I, Chapter X, the pine-thistle and "all the plants which belong to that class," the ἀκανθῶν or ἀκανθωδῶν. As he mentions the Acanaceæ again more than once it is obvious that the recognition of the Cynareæ as a group was to Theophrastus a commonplace. In Book I, Chapter XIII, he also describes a group of plants distinguished by having the flower on the top of the actual seeds and with one flower attached to each seed; as these

are described as "all thistle-like plants" it seems tolerably certain that in addition to Cichorieæ and Cynareæ this perspicacious Greek recognised the Compositæ as a class. The only other large obviously natural family which corresponds to this definition is the Umbelliferæ and this was separated as the Ferulaceæ.

Dioscorides. Circa A.D. 65.

According to Smith's Classical Dictionary, Pedacius Dioscorides probably lived in the second century A.D., but Burgess (16) gives evidence for a date about 65 A.D. for the writings of this author; Adanson (1) gives 50 A.D. as the date. The variations in the different editions of his *Materia Medica* are notorious but as early as 1555 the grouping of *Sonchus*, *Cichorium*, and *Chondrilla* by Dioscorides was amplified in the annotations of a Castillean edition by Dr. Andrés de Laguna (23) who noted the similarity to the above-mentioned genera of *Scariola*, *Picris* and *Taraxacum*.

In the edition of 1598 by Bauhin of Matthioli's Dioscorides (24) other groupings of Composites are to be noticed, thus Lib. III, Cap. XIX, deals with *Leucantha*, identified as *Carduus Mariæ* by Bauhin and the subject of Cap. XXI is identified with *Carduus stellatus*. Chapter 109 of the same book deals with *Tussilago Farfara*, 110 and 111 with forms of *Artemisia* and 112 with *Ambrosia*; similarly *Anthemis*, *Parthenium* (= *Matricaria*) and *Bupthalmum* (*Bellis*, according to Bauhin) are dealt with in chapters 137-139. It is possible that this grouping of *Anthemis* and *Matricaria* forms the first indication of the Anthemideæ and this is rendered more probable by the development of the group by Brunfels.

16th Century.

Following upon Dioscorides comes a gap in the history of botany in general of about fifteen centuries. During the 16th century various commentators on Dioscorides, such as Brunfels (15), Valerius Cordus (20) and Fuchs (31) established and extended the Anthemideæ while retaining the Cichorieæ and Cynareæ. Lobel (60) extended these three groups and classed a few genera of the Astereæ together. Caesalpino (17) was the first to group the Compositæ as a whole. His "Herbaceæ pluribus seminibus" are divided into the "Anthemideæ" and "Cichoraceæ aut Acanaceæ." The "Anthemideæ," however, include all the Composites not in the Cynareæ or Cichorieæ, while the Anthemideæ as recognised by the earlier writers is considerably obscured.

17th Century.

Bauhin (6) fails to recognise the Compositæ as a family but retained the groups previously recognised, i.e., the Cichorieæ,

Cynareæ, Anthemideæ and Astereæ, and in addition he classed several of the genera of the Inuleæ together. A decade later Gerard (33) advanced still further with a suggestion of the Calenduleæ as a group and the consolidation of the Inuleæ in addition to the groups of Lobel.

In the latter half of the century Morison (65) and Ray (72-73) again recognised the Compositæ as a family and agree in retaining the Cichorieæ and Cynareæ while confusing all the other tribes. The remaining authorities of the 17th century, such as Christopher Knaut (48), Rivini (75), Hermann (39) and Tournefort (84) agree in regarding the Composites as a family with the Cichorieæ as a distinct section, but confuse the other tribes. Knaut seems to be the first to use the name Compositæ, Ray's designation is "Composito flore." Hermann classes the group as "Gymnomonospermæ Compositæ."

18th Century.

The dominating systematist of this century, was, of course, Linné, but in his three attempts (59) at the classification of the Compositæ he made no progress whatever. Indeed, his first arrangement included so many external genera, such as *Globularia*, *Protea* and *Leucodendron* that he can scarcely be said to have recognised the family at all. His "Syngenesia" in the Sexual System include *Lobelia*, *Viola* and *Impatiens*! The *Ambrosia* group are separated for the first time in his Natural System.

Linné was preceded by a group of systematists whose ideas on the Compositæ varied very much. Boerhaave (12) gives the character "staminibus propriis coalitis in tubum" for the first time and has one section "semine âculeis donato" which foreshadows the Heliantheæ. Christian Knaut (49), Ruppianus (76), Magnol (62), Ludwig (61), Allionius (2) and Necker (66) made attempts somewhat similar to the systems of Tournefort and Rivini; in some cases the presence or absence of rays was used as a diagnostic character.

The first botanist to consider the Compositæ alone was Vaillantus (85) whose system bears a distinct resemblance to that of Boerhaave, with an amplification of the Heliantheæ. Pontedera (71) gives a system very similar to that of his contemporary, Vaillantus, but with suggestions in his grouping of the genera of several tribes such as the Senecioneæ, Calenduleæ, Astereæ and Helenieæ, which were not recognised by that systematist. Berkhey (9) and Meese (64) also published dissertations on the Compositæ but these show little or no originality.

Adanson (1) reviewed previous systems and places the Campanulaceæ next the Compositæ. He recognised some of the previously distinguished tribes (vide Table I) and followed Linné in separating the *Ambrosia* group but retained it within the family. Jussieu (46) makes the "antheræ connatæ" a diagnostic character of the family and foreshadows the Arctotideæ and Mutisieæ in his Cinarocephalæ anomalæ. In his arrangement of the genera Gaertner (32) follows Pontedera in the characters (receptacle, pappus and ray) used and arrives at a similar result, recognising the Inuleæ and Anthemideæ in addition to Pontedera's groups.

Early 19th Century.

In the beginning of this century Richard (74), Batsch (5) and Lagasca (51) followed their predecessors with little or no originality in the Compositæ, except that Rich used the divided or undivided style to characterise his two sections of the Synantheria.

Cassini, 1813-1834.

The founder of synantherology and the master in whose footsteps all subsequent students of the Compositæ have followed was Henri Cassini. As the result of a systematic analysis of all the floral characters he arranged the genera of the family into a series of 20 tribes with sections and sub-sections (vide 18, Vol. III, Synopsis) which have been retained as units during the various minor changes made by later systematists. There is no detail of morphology which escaped the notice of Cassini, and the present intensive study of the family by including physiology and geographical distribution can modify only slightly the grouping of the tribes. Even then Cassini had already indicated the existing relationships which will be emphasised later.

Cassinian Period, 1820-1917.

There have been three chief modifications of Cassini's system. Lessing (55) by using only the style characters reduced the number of tribes to eight but retained practically all Cassini's sub-tribes. De Candolle (21) followed Lessing with the addition of many original divisions of the sub-tribes. Bentham (8) returned to Cassini's system because he appreciated the value of the characters of the stamens and he acknowledges this return (7) but claims to have arranged the family before he was aware his system was so similar to that of Cassini. The present system is, therefore, one reached independently by the only two botanists who ever studied the family sufficiently thoroughly to be able to speak with authority.

Other systematists follow these four more or less closely: Bartling's system (4) is frankly Cassinian; Link (58) followed Cassini with several retrogressive variations; Lindley neglects Cassini and Lessing, returning to Jussieu (56) and later (57) following De Candolle. Endlicher (29), A. de Jussieu (47) and Eichler (27-28a) all follow De Candolle. Payer's arrangement (70) is a curious atavistic return to the early 18th century and Baillon (3) fuses several pairs of Benthamian tribes. Hoffmann's arrangement (42) shows no originality. One or two obvious sub-tribes are sunk and minor suggestions made by Bentham are carried out. Hoffmann gives an artificial sub-division of the Cichorieæ, but Engler and Gilg (30) returned to the Benthamian arrangement. Wettstein (89) follows Hoffmann.

Floras.

The Floras of any given period usually follow the current system. Thus Meese (63) and Nuttall (69) follow Linné; Kunth (50) follows Cassini with slight variations; Gray follows first De Candolle (34-35) and then Bentham (36). Boissier (13) follows Bentham.

American Systems.

The Americans show a tendency to raise the status of the groups: thus Jepson (45) raises the Ambrosinæ and Madinæ to the rank of tribes; Britton and Browne (14) raise the Ambrosinæ and Cichorieæ to the rank of families; J. K. Small (79) follows Britton and Browne, and Bessey (11) raises all the tribes and the Ambrosinæ to the rank of families, making fourteen in all.

Special Memoirs.

Don (25) and Schultz-Bipontinus (78) attempted classifications of the Cichorieæ. Nees (67) and Burgess (16) have dealt with the Asters and Delpino (22) proposed a new sub-division of the Senecionideæ of Lessing, but these isolated memoirs have had no effect on the general classification of the family.

Conclusion.

From Table I it will be obvious that the history of the classification of the Compositæ is clearly divided into two epochs, pre-Cassinian and Cassinian, with little or no advance from Theophrastus until the few decades immediately preceding Cassini, in which some slight indications are given of the origin of the ideas of a few of the tribes afterwards defined by the master. The half century following Cassini was marked chiefly by the burying of his classic memoirs by Lessing and De Candolle and the present Benthamian period is nothing more than a return to the teaching of the greatest of all synantherologists.

TABLE I.

Tribes recognised from Theophrastus to 1915.

Date.	Author.	Compositæ.	Vernonæ.	Eupatoriæ.	Asteræ.	Inulæ.	Helianthæ.	Heleniæ.	Anthemidæ.	Senecionæ.	Calendulæ.	Arctotidæ.	Mutisiæ.	Cynaræ.	Cichoriæ.
B.C. 320	Theophrastus	+												+	+
A.D. 65	Dioscorides								?					+	+
1532	Brunfels								+					+	+
1540	Cordus								+					+	+
1542	Fuchs								+					+	+
1570	Lobel				+				+					+	+
1583	Caesalpino	+							?					+	+
1623	Bauhin				+				+					+	+
1633	Gerard	?			+	?			+		?			+	+
1680	Morison	+												+	+
1682-86	Ray	+												+	+
1687	Knaut (1)	+												?	+
1690	Rivini	+												?	+
1690	Hermann	+												+	+
1700	Tournefort	+												+	+
1710-20	Boerhaave	+					?							+	+
1716	Knaut (2)	+												?	+
1718	Ruppius	+												+	+
1718-21	Vaillantus	+					+	?						+	+
1720	Pontedera	+			?		+	?		+	+			+	+
1720	Magnol	+												?	+
1737	Ludwig	+												?	+
1736-38	Linné	?												?	?
"	"	+					?							?	?
"	"	+*												?	?
1757	Allionius	+												+	+
1760	Berkhey	+												+	+
1760-61	Meese	+					?			?				?	+
1763	Adanson	(+*)					+							+	+
1789	Jussieu	(+*)					+			+		?	?	+	+
1791	Gaertner	(+*)			+	?	+	+	+	?	?			+	+
1791	Necker	+					?		?					+	+
1801	Rich	+			?	?				+				+	+
1802	Batsch	+								?				+	+
1816	Lagasca	+					+							+	+
1818	Nuttall	+					?							?	+
1813-34	Cassini	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1820	Kunth	+	?	+	+	?	+		+	+			+	+	+
1829	Link	(+*)	+	+	+	?	+		+		?		+	+	+
1830	Bartling	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1832	Lessing	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1830-36	Lindley	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1836-38	De Candolle	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1836-40	Endlicher	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1860	Payer	+*								+				+	+
1873	Bentham	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1875	Boissier	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1875-80	Eichler	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1882	Baillon	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1831	Gray	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1857	"	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1884	"	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1897	Hoffman	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1898	Britt. & Brown	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1901	Wettstein	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1901	Jepson	(+*)	+	+	+	+	+	+	+	+	+	+	+	+	+
1903-13	Small, J. K.	+*	+	+	+	+	+	+	+	+	+	+	+	+	+
1915	Bessey	+*	+	+	+	+	+	+	+	+	+	+	+	+	+

+ Family or tribe recognised as a group.

+* Ambrosiaceæ as a distinct family.

(+*) Ambrosiaceæ as a tribe or sub-tribe.

B. PHYLOGENY.

Beyond vague ideas of a common ancestry for some of the genera obviously very much alike, the pre-Cassinian writers on the Compositæ had apparently no thoughts of the evolution of one group from another within the family. It is, of course, well known that many of them were vehemently opposed to all evolutionary doctrines. As this question of creation or evolution is a general one and as Cassini was the first to distinguish the tribes sufficiently clearly to form any correct idea of their relationships the present account will be given in chronological order from Batsch and Cassini onwards. Another reason for the chronological order is that it emphasises the penetration of Cassini and the subsequent neglect of his valuable work by succeeding writers to the great detriment of synantherology.

Batsch, 1802.

In his *Tabula affinitatum* (5) Batsch discusses the affinities of each of his three groups of Composites. He considers the Lepid-ocephalæ (Cichorieæ) to be connected with the Cinarocephalæ (Cynareæ and Mutisieæ) through *Scolymus carduiiformis* and *Barnadesia*, with the Campanulaceæ by the aggregation of the flowers and by the latex and with the Cucurbitaceæ by the bitter juice and disagreeable odour. The Cinarocephalæ he considers to be connected with the Valerianaceæ and Proteaceæ by the exalbuminous seed and aggregation of the flowers; similarly the Corymbiferæ are said to have affinities with the Campanulaceæ.

Cassini, 1826.

The form chosen by Cassini for the expression of his views on the affinities of the tribes of the Compositæ is one seldom used but very useful. In Plate I of the *Opuscules* (18) he places the nineteen tribes each in a circle and the circles in an ellipse. Fig. 1 is from Cassini's diagram in the *Opuscules*, which he describes as a "tableau exprimant les affinités des tribus naturelles de la famille des Synanthérées." The Boopidéæ are the Calyceraceæ of modern systems. The interesting points in relation to the present study of the Compositæ are the affinities indicated between the Senecioneæ and the Eupatorieæ, the Senecioneæ and Astereæ, the Senecioneæ and Anthemideæ, the Senecioneæ and Mutisieæ, and between the Inuleæ and Cynareæ.

Lessing, 1832.

On page 435 of the *Synopsis* (55) Lessing gives a table of the tribes and sub-tribes of which he remarks "Analogiam subtribum singularum videre licet e tabula sequente." An examination of this arrangement (Table II) shows that Lessing followed Cassini in his

TABLE II. Lessing's scheme of relationships of the tribes of Compositæ.

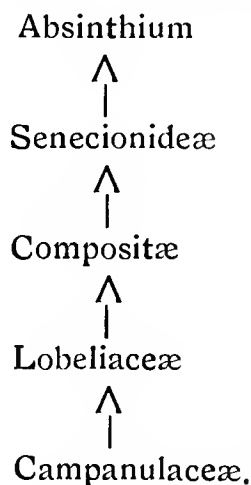
I	II	III	IV	V	VI	VII	VIII
Cynaroideæ	Mutisiaceæ	Cichoriaceæ	Vernoniaceæ	Eupatoriaceæ	Asteroideæ	Senecionideæ	Naussauviaceæ
Arctotideæ	Mutisiæ		Liabæ Pectideæ		Astereæ	Senecioneæ Tagetineæ Heleniææ Chrysanthemeæ	
Centaureiæ							
Xeranthemæ							
Calenduleæ					Inuleæ	Leysseriæ	
Othonnæ				Tussilaginéæ	Baccharideæ	Artemisiæ	
	Facelideæ Leriæ				Tarchonanthæ	Gnaphalieæ	
					Bupthalmæ	Relhanieæ	
					Ecliptæ	Heliantheæ Flaveriæ	
			Trichospireæ	Alomieæ	Melampodieæ	Ambrosiæ	
Echinopsideæ Cardopateæ			Rolandreæ Elephantopodeæ				Nassauviæ
Carduineæ		Lactuceæ Hieraciæ Scorzonereæ Hypochoærideæ Hyoserideæ	Vernoniæ	Eupatoriæ			Trixiæ
		Lampsaneæ Scolymeæ		Agerateæ			

placing of the Othonneæ in the Cynaroideæ and the Tussilagineæ in the Eupatorieæ marks the affinity of the Benthamian Senecioneæ with these two tribes, as the Othonneæ and Tussilagineæ now form sub-tribes of the Senecioneæ. His grouping of the Inuleæ and Leyssereæ, Tarchonantheæ and Gnaphalieæ, Buphthalmeæ and Relhanieæ is interesting as all these are sub-tribes of the Inuloideæ of Bentham. Various other groupings are of minor interest but will be obvious to the synantherologist.

Delpino, 1871.

The stress laid upon the anemophily in *Artemisia* by Delpino (22) has been shown by Bentham (7) to have led to erroneous conclusions. His views on the origin of the family as shown in Table III are interesting on account of the recognition of the affinity between the Lobeliaceæ and Compositæ. It is necessary to remember however that his Senecionideæ is that of Lessing and includes the Heliantheæ, Helenieæ and Senecioneæ.

TABLE III. *Delpino's scheme of the derivation of Absinthium.*



Bentham, 1873.

Bentham followed Cassini very closely in his expression of the affinities of his tribes, as will be seen from Fig. 2, which is the arrangement given in Plate II. of the *Notes* (7). This diagram fails to indicate the close affinity of the Senecioneæ and the Eupatoriaceæ through the Tussilagineæ, recognised by Cassini and certainly not obliterated by the removal of that subtribe from the latter to the former tribe by Bentham. Similarly it omits the connection between the Cynareæ and Inuleæ noted by Cassini. It marks clearly, however, the affinities of the Senecioneæ with six other tribes.

In addition to the above diagram Bentham gave expression to other views on the history of the family in the text of the *Notes*

(pp. 481 sqq.). He considers the Helianthoidæ to be the most ancient tribe on account of the foliaceous involucre bracts, aristate pappus and free anthers of some genera. From the ancestral Helianthoidæ by the regular development of a tubular corolla and the substitution of a pappus for a reduced calyx he supposes the Eupatoriaceæ arose in America, the Vernoniaceæ in the New and Old Worlds, the Cynaroideæ in the northern and the Mutisiaceæ in the southern hemisphere. From the same plexus, by a reduction of the corolla, a disappearance of the stamens and an oblique development of the corolla in the outer florets, he supposes the modern Helianthoidæ arose in both West and East, and also the Helioidæ in the former hemisphere and the Anthemideæ in the

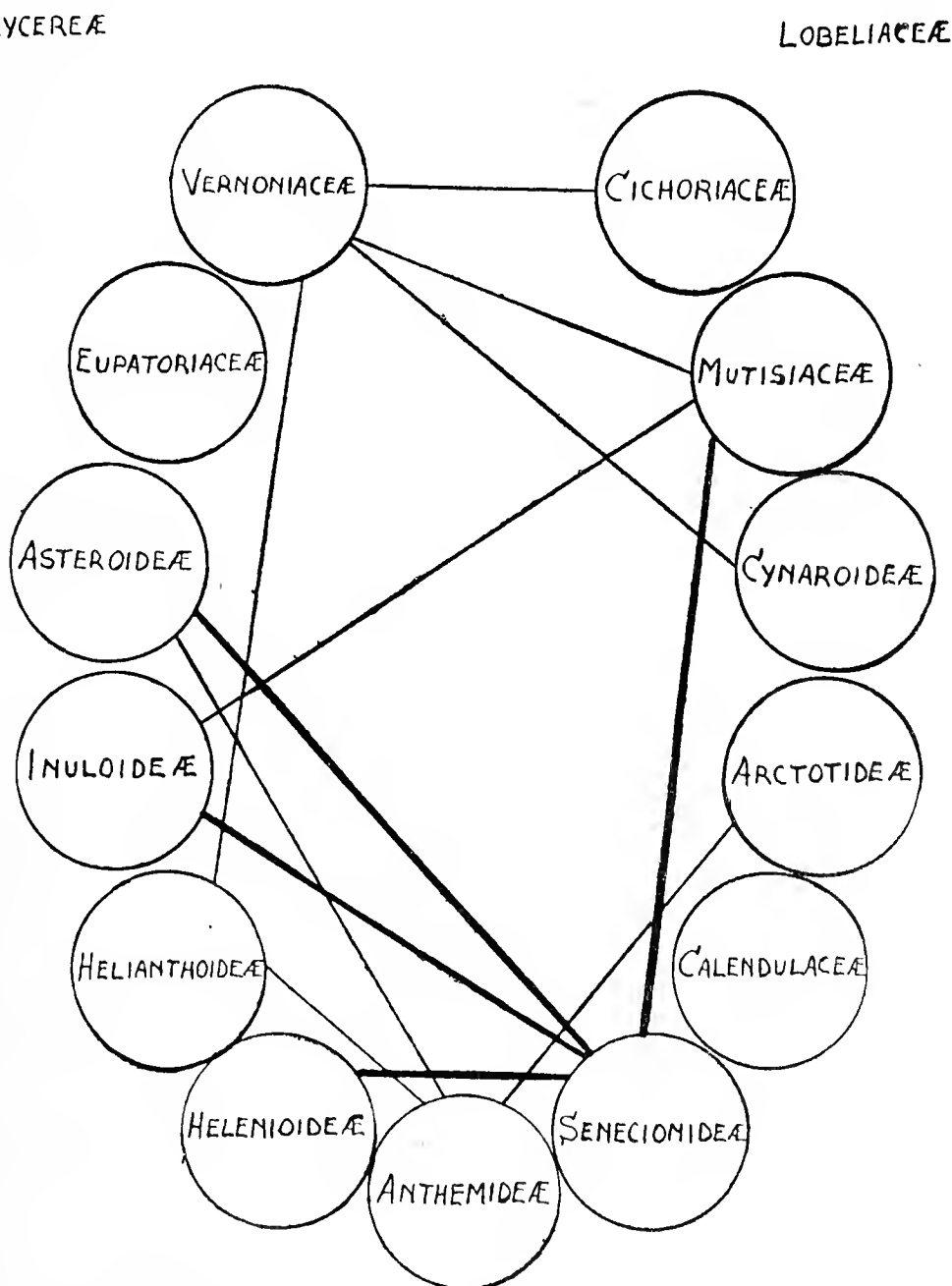


FIG. 2. Bentham's scheme of relationship of the tribes of Compositæ.

latter. By reduction in the involucre and development of a setose pappus the Asteroideæ, Senecionideæ and Inuloideæ are said to have arisen in both Old and New Worlds from this Helianthoid plexus. Finally by a unilateral development of all the corollas, a reduction in the involucre and the development of a pappus he suggests the Cichorieæ arose also from the ancestral Helianthoideæ. It will be noticed that these opinions scarcely coincide with those expressed in the diagram (Fig. 2), and they will be discussed later in the light of the present investigation.

Vuillemin, 1884.

The most comprehensive study of the anatomy of the Compositæ is Vuillemin's *Tige des Composées* (86), an investigation "de la valeur des caractères anatomiques au point de vue la classification des végétaux." He concluded that although anatomical characters may be of value in the identification of fragments of plants such as drugs and fossil debris or in indicating the affinities of species within a genus or in special cases even genera within tribes, they were of no value as a basis for classification in the larger groups of the Compositæ.

Hildebrandt, 1887.

Vuillemin's conclusions were controverted three years later by Hildebrandt (40), who, however, confined his investigations to the Ambrosiaceæ and Senecionidéæ (of Lessing). He reclassified the genera he examined on the anatomy of the stem, but his subsections as he characterises them show many variations in their anatomy, and there is an example of almost each type of structure in most of his subtribes.

Nichols, 1893.

After studying the achenial hairs of the Compositæ Nichols (68) decided that they were of no use as tribal characters. "Within the genus, however, the characters seem to be more constant, and might, perhaps, be made of value in the determination of species."

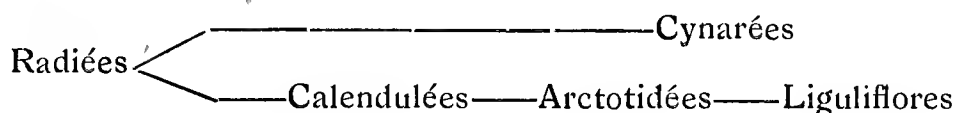
Höck, 1898.

Dealing with orders and not with families Höck (41) derives the Campanulatae from a line showing epigyny; the main line gives a Rubialian line near the base from which a line leading to the Umbellifloræ is given off, the main line culminating in the Campanulatae.

Col, 1899.

From his study of the laticiferous tissue and other secretory

canals in the Compositæ Col (19) considered that the Cynareæ had arisen from the "Radiées" and that the Calenduleæ, Arctotideæ and Cichorieæ were developed on a line of evolution parallel to that which gave the thistles. His views are expressed thus:—



Hallier, 1905.

The Campanulatae according to Hallier (38) came from the Gymnosperms via the Polycarpicæ, Magnoliaceæ, Aristolochiaceæ and Passiflorales. He discusses briefly the Campanulaceæ, Calyceraceæ and Compositæ, considering the last to be the highest expression of several of the tendencies shown by the Campanulatae.

Lavialle, 1912.

After an elaborate and extensive investigation of the development and structure of the achene Lavialle (52) gave expression to an opinion upon the affinities of the Cichorieæ, Cynareæ and Mutisieæ. The examination included 298 species, 65 genera and most of the subtribes in the above-mentioned groups. Lavialle did not, however, extend his observations to the rest of the Compositæ so far as I have been able to ascertain at present. His views are given in the form of a diagram, Fig. 3, but their value is

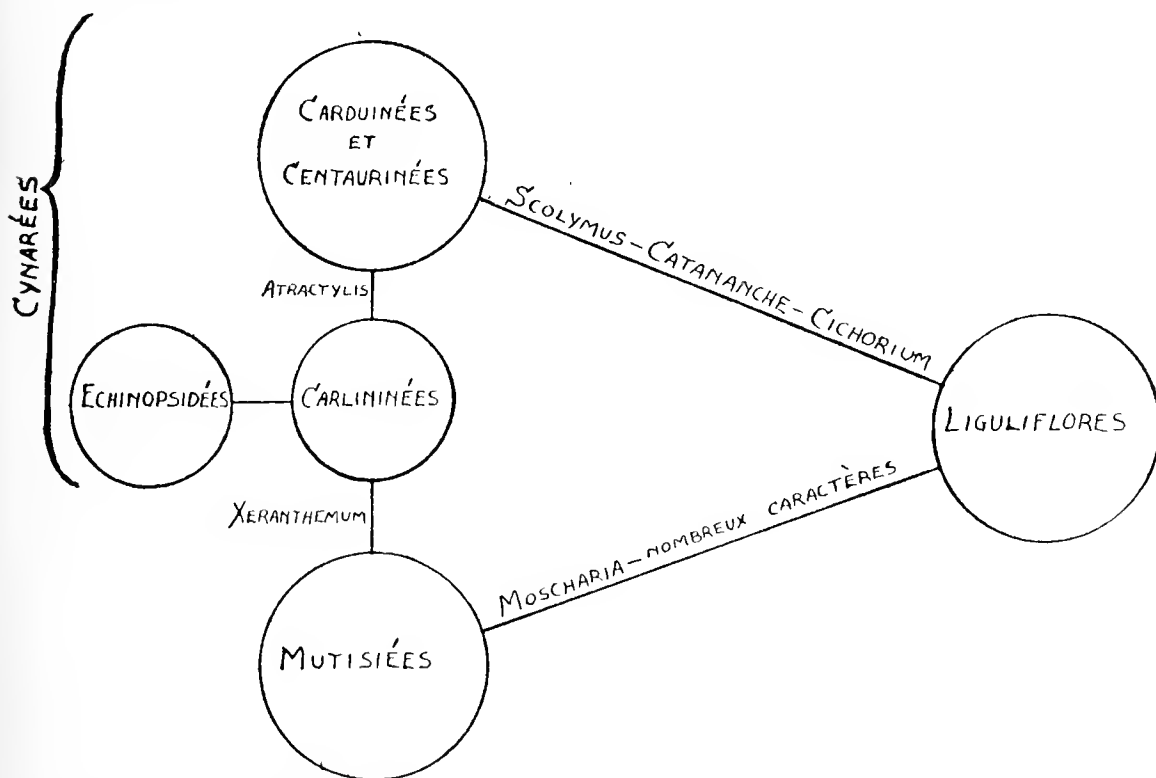


FIG. 3. Lavialle's scheme of the affinities of the Mutisieæ, Cynareæ, and Cichorieæ.

somewhat reduced by the limitation in the number of tribes examined.

Wernham, 1913,

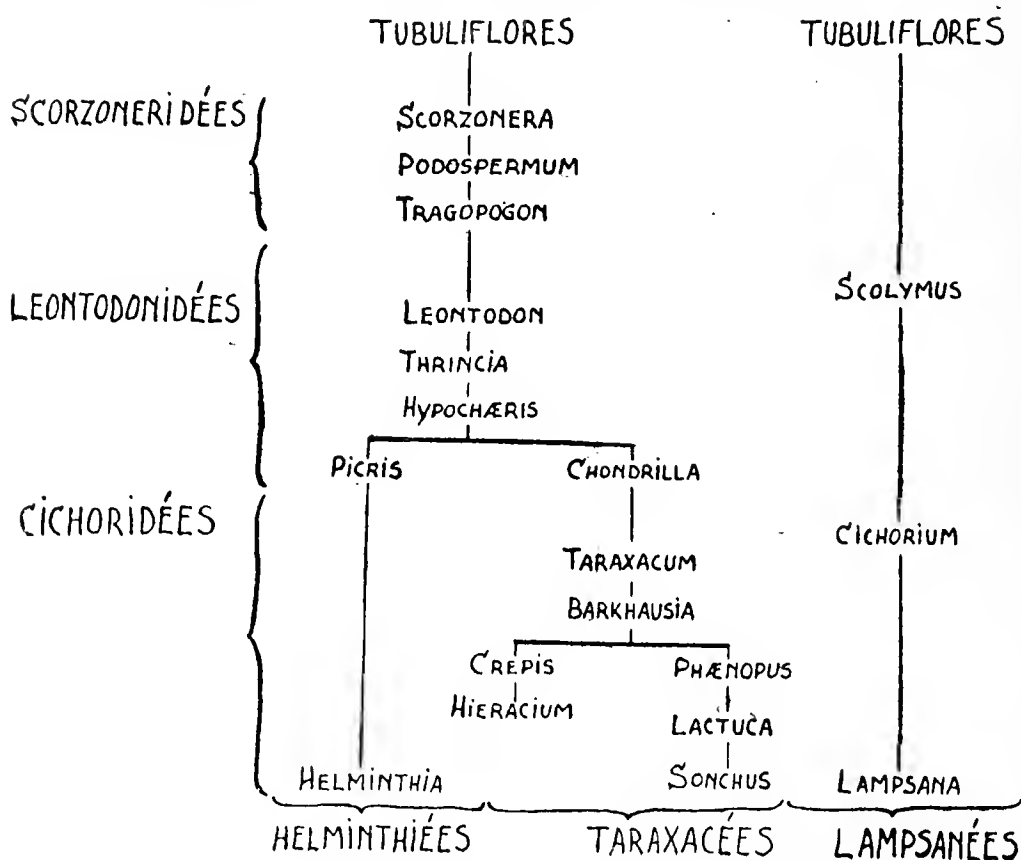
In his account of floral evolution in the Sympetalæ Wernham (88) does not consider groups below the rank of family but he supports the view that the Compositæ have arisen from the Campanulacæ, an affinity which was recognised early in the history of the two families. The most important of Wernham's contributions is contained in the section on the determination of plant affinities in his "Summary and Conclusion" to which reference will be made later.

Dufour, 1907, and Lebard, 1913.

These two investigators (26 and 53) laid considerable stress upon the form of the cotyledons in the Cichorieæ, and Dufour proposed that the tribe should be divided on this character into two subtribes, the Brachycotylées (type *Lactuca* with short, broad cotyledons) and the Leptocotylées (type *Scorzonera* with long, narrow cotyledons). Dufour's work was carried further by Lebard, who distinguishes a form of cotyledon intermediate between the short and the long forms and gives a phylogenetic diagram shown in Table IV.

TABLE IV.

Lebard's phylogenetic scheme of the Cichorieæ.



He regards the Leptocotylées as primitive and the Brachycotylées as derived along two lines of evolution from the Tubulifloræ.

Lee, 1914.

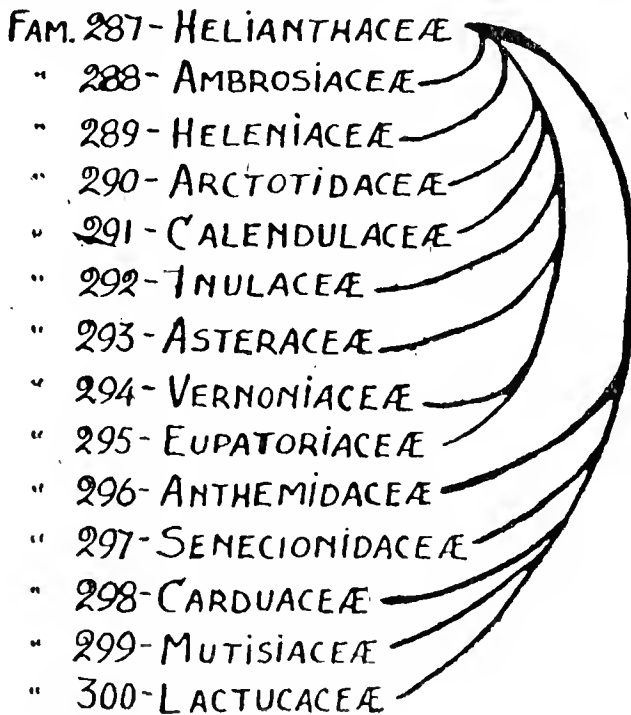
After an investigation of the seedling anatomy of about 50 species of Compositæ belonging to most of the tribes Lee (54) concluded that the classification in this family could receive no assistance from seedling anatomy. As he found all the types of anatomy which occur in the family in closely related species, and even in different specimens of the same species it is obvious that he was right in his conclusion that seedling anatomy is of no value in dealing with questions of affinity within the Compositæ. This is one more example of the failure of anatomy to give phylogenetic characters in the Compositæ, and agrees with the facts given by Vuillemin and Hildebrandt, although not with the latter's conclusions.

Bessey, 1897, 1915.

In his presidential address to the Botanical Society of America in 1897 (10) Bessey placed the Asterales at the summit of the Rubialian line of evolution. The four families included in the Asterales were supposed to be derived from one another thus:—Valerianaceæ—> Dipsaceæ—> Calyceraceæ—> Compositæ. The same author in 1915 (11) enunciated various dicta for determining

TABLE V.

Bessey's phyletic lines for the tribes of the Compositæ.



the phylogeny of flowering plants. These dicta, however, apply only to certain groups in each case. He raises the fourteen tribes of the Compositæ or Asterales to the rank of families, and regards the Helianthaceæ as the primitive group from which two principal phyletic lines have arisen, culminating in the Eupatoriaceæ on the one hand and the Lactucaceæ on the other. His views may be expressed as in Table V.

Hutchinson, 1916.

The Heliantheæ are also regarded as the most primitive tribe by Hutchinson (43) who considers the aristate or paleaceous pappus and the receptacular paleæ as primitive characters, and who also considers (44) the structure of the pappus in the Compositæ as "perhaps one of the most important features in the consideration of the phylogeny and affinities of the genera of this interesting family." This is very different from Bentham's view of both pappus and receptacular paleæ, the inconstancy of which in genera and sections led to that synantherologist to consider these characters as of use only when supplemented by other and more constant features.

Small, 1915-16.

The present writer has made various suggestions as to the affinities of the Compositæ and its constituent tribes. The first (80) was made after a study of the variation in styles and stamens and the elucidation of the function of the appendages of these organs. The chief point made was the establishment of similar lines of evolution from the consideration of the styles and of the stamens. As the work along these lines has been revised and extended it will be enough at this juncture to note that the Senecioneæ were considered the primitive group. In a subsequent study of the corolla (81) it was suggested that the Cichorieæ arose directly from the Senecioneæ by a comparatively small mutation. Other observations on the displacement and multiplication of the ovules in *Senecio vulgaris* (82) controverted the removal by Warming (87) of the Calyceraceæ from the neighbourhood of the Compositæ. These studies have been continued, and the present account is the result of the extended investigations.

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RHIZOPHIDIUM ACUFORME (ZOPF) FISCH.

BY W. B. GROVE, M.A.

[WITH ONE FIGURE IN THE TEXT].

ON *Chlamydomonas intermedia*, of which a large gathering was made at Harborne (in the same cart-rut that is mentioned in the following note), there was found in April, 1917, a quantity of *Rhizophidium acuforme*. This parasite has been previously recorded by Zopf on a *Chlamydomonas*-like organism in March from an ice-covered pond in Pomerania, but there remained a doubt how far it was really distinct from *R. globosum* (Braun), which occurs on a large number of fresh-water Algæ. This doubt is dispelled by the present find.

The cells of the *Chlamydomonas* are attacked by the zoospores of the *Rhizophidium*, while still in active motion, at any point of their circumference; one to four (or even five or six) may be

found on the same individual. After attaching itself to its victim, the zoospore surrounds itself with a membrane, pierces the thin cell-wall of its host, and sends a short and slender mycelial tube through it into the cell; this tube often enlarges slightly just below the puncture to form an appressorium which holds it securely to the prey. The tube then grows further and sends out a few short stoutish branches which spread over the surface of the protoplast and act as haustoria; no threads could be detected within the protoplast.

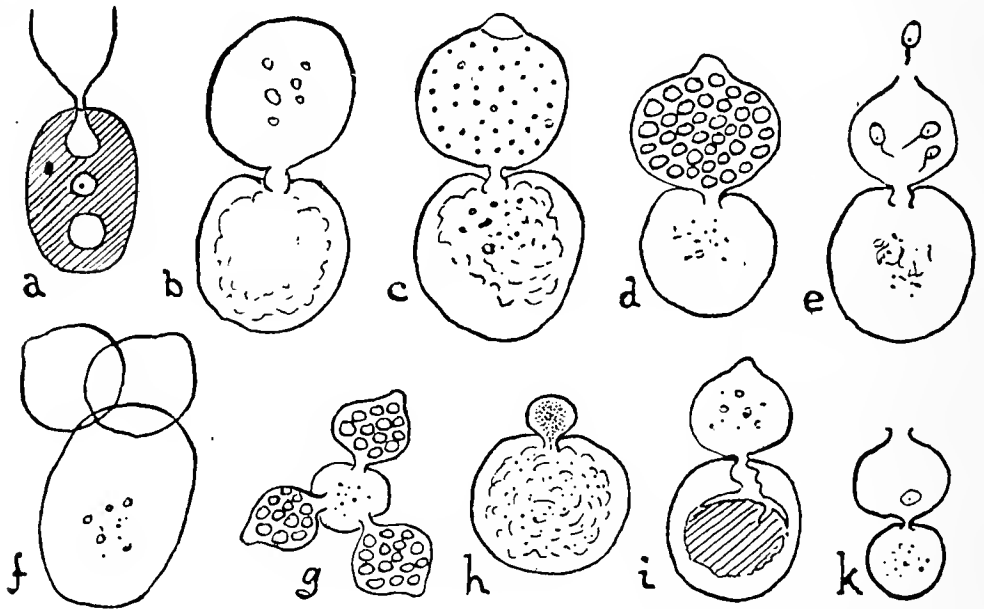


FIG. 1. *RHIZOPHIDIUM ACUFORME* ON *CHLAMYDOMONAS*.

- a. The *Chlamydomonas* in health.
- b. The *Chlamydomonas* with a zoosporangium as large as itself attached.
- c. Formation of the beak and oil-globules.
- d. Formation of zoospores.
- e. Escape of the zoospores.
- f. A *Chlamydomonas* with two empty zoosporangia,
- g. Another with three sporangia full of zoospores.
- h. With a young zoospore just attached, plasmolysed with iodine.
- i. Showing the mycelium with branched haustoria, attached to the protoplast.
- k. An evacuated sporangium, containing one dead zoospore, and showing the curled back edges of the pore. (All magnified 1000 times.)

At first the zoospore is rather less than 2μ in diameter, and oval or nearly round in shape; it grows rapidly, remaining the while all but spherical, until if there is only one on the *Chlamydomonas* it becomes as large as its host, *i.e.*, it may reach a diameter of about $15-16\mu$, the host measuring on the average $20 \times 12\mu$. During growth it is filled with a clear colourless protoplasm, which can only be seen by plasmolysing: in this are often embedded a few (1-6) relatively large round oil-guttules. Meanwhile the protoplasm of the *Chlamydomonas* has withdrawn from its wall and collected into an irregular mass which is being

gradually absorbed through the mycelium. Finally there is left of it nothing but a few reddish granules, of nearly the same colour as the eye-spot of the *Chlamydomonas* when it was in health.

Towards the end of growth the thin wall of what has now become the zoosporangium of the *Rhizophidium* bulges out at the summit into a little papilla of mucus which is surrounded by a definite circular margin: the mucus evidently arises by the conversion of a determinate area of the cell-wall into a substance which swells up in water. There are by this time formed within the sporangium a number, 20-50, or it may be 50-80 (according to its size), of little rounded masses, each enclosing a minute oil-drop. These are the zoospores, which arise in the following way. At first, as has been said, only a few scattered oil-drops are seen in the clear protoplasm, these then increase in number, becoming smaller as they do so, and each is surrounded by a little mass of protoplasm which can be brought into distinct view by running in iodine solution. When these are mature, the papilla has reached its greatest size, and the zoospores begin to escape, a few at a time or more slowly one by one. The single flagellum trails behind and the oil-drop occupies the rear position. When the number of zoospores in a sporangium is reduced to a few, it is interesting to observe the remainder as they escape very slowly, often with an interval of several (15-20) minutes between each. Those left move freely about in a jumpy way, and one occasionally will thrust its head into the mass of mucus and push or wriggle in its efforts to get through: these may continue for several seconds and then succeed, or the zoospore may give up the attempt for a time and continue its wanderings within the sporangium, though it returns again and again persistently to the place of exit. On emerging the zoospore usually travels two or three times the length of the sporangium away, and then rests for a few moments before beginning to travel in a curvilinear course varied with a series of hops and skips.

In the free state the zoospores can be seen threading their way among a mass of *Chlamydomonads* like a number of sight-seers promenading down Oxford Street and gazing into the shop windows on either side, or rather they are more like a crowd of farmers up from the country at a Cattle Show, passing irregularly from pen to pen and choosing which animal they shall buy. Similarly the zoospore seems to inspect the *Chlamydomonads*, as if selecting its victim. After it has fastened upon one no power is left to its prey to shake off the burden, though the *Chlamydomonas* still

swims freely about, even after the sporangium has grown nearly as large as itself. To see the two in that condition, twisting, turning, doubling, backing, rolling over one another, reminds one of the gambols of two dogs fighting in a half-friendly way in a field, or of two equally matched boys wrestling upon a lawn. The *Chlamydomonas*, judging by its antics, is evidently excited and uncomfortable under the infliction, but at length it succumbs and becomes motionless: its protoplasm disintegrates and discolours, the sporange reaches its full size, the beak develops and the zoospores begin to escape through the mucus. When all are gone, the mucus has disappeared; the opening, which is often wider than a zoospore, is clearly seen, its edges curling back and giving the empty sporange an urn-like shape. The complete evacuation may occupy a period of more than an hour or two.

During all the time of observation no resting spores were seen, nothing but zoosporangia, and all these were of exactly the same character. Dangeard ("Le Botaniste," i., 61, pl. 3, Fig. 12-15) reported *R. globosum* as occurring on *Chlamydomonas*; the account given agrees in its details with those of *R. acutiforme* as here described, except that the sporangia are said to allow the zoospores to escape through four or five little holes, which would thus bring the parasite under *R. globosum*. Nothing approaching this was seen in the present instance; in every one of the hundreds of cases observed the single opening was always exactly apical.

NOTE ON *PLEODORINA ILLINOIENSIS*.

In June, 1915, I recorded in the NEW PHYTOLOGIST (XIV, p. 169) the finding of *Pleodorina illinoiensis* during the preceding spring in cart-ruts at Harborne, Warwickshire. The alga occurred, in small quantity, in the same unchanged ruts in 1916, and again in larger quantity in April, 1917. But this year it was accompanied by about equal quantities of *Pandorina Morum* and *Eudorina elegans*, as well as by other Algæ. Moreover, although the elliptical form and the posterior protuberances were as marked as ever (or the latter even more so), there was intermixed a greater number of transitional stages between it and the *Eudorina*. Therefore the suspicion expressed in 1915, that this *Pleodorina* is merely a well-marked mutation of *Eudorina*—on the way to becoming a species, it is true, but not yet completely settled down—is entirely justified. This is another instance, added to the many already known but persistently ignored in certain quarters, in which the evolution of a species, or even of a genus, is taking place before our eyes.

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OBSERVATIONS ON THE INFLUENCE OF AERATION
OF THE NUTRIENT SOLUTION IN
WATER CULTURE EXPERIMENTS, WITH SOME
REMARKS ON THE WATER CULTURE METHOD.

BY WALTER STILES AND INGVAR JÖRGENSEN.

GENERAL INTRODUCTION.

INVESTIGATIONS dealing with the growth of plants in water cultures have been pursued now for more than two hundred years since Woodward carried out his experiments at the end of the seventeenth century. During that time such studies have passed through various phases, from the first crude observations to the more definite experiments which resulted from the development of the water culture method after the successful part it played in the controversy regarding the essential elements for plant nutrition and growth. The technique of the water culture method as it is employed at present was chiefly developed by Sachs. Later investigators have introduced various slight alterations in the technique, but in its broad principles the water culture method remains today much the same as Sachs left it. It is, in short, included in the general stagnation which has characterised plant physiology during the last fifty years.

The essential of the water culture method has been to give a plant a nutrient solution of a definite composition and after an arbitrary time to measure the dry weight of the plant. There is no attempt to control any factor except that of the nutrient medium surrounding the roots, or to take account of any process beyond that of growth as measured by increase of dry matter.

Such methods should not be used without reference to the other factors influencing the life of the plant, or without examining how the life of the plant is influenced in its various phases. Our whole outlook on the physiology of the plant, resulting as it does, from

incomplete researches on isolated organs and imperfect generalisations derived therefrom, fails to realise the plant as a unit, or to consider it as a whole. Thus the law of the minimum and the special case of it called the principle of limiting factors are not of general application to the activity of the plant as a whole on account of the interaction of factors and the processes dependent on them. We are still waiting for the more exact statement which shall properly express the law of physiological relations—in other words, we are still waiting for a more satisfactory primary survey of the life of the plant. We are waiting, in fact, for that science of botany which will embody physiology, ecology and agriculture and make it the science of the living plant as a whole, having as its basis an elementary analysis of the plant's activities in relation to environmental and hereditary factors.

How little this broader outlook appears in the water culture method is apparent at once. We give a supposed definite composition of the nutrient solution and correlate this with the dry weight of the plant after an arbitrary time. There is no notice taken of alterations *with time* in the water culture solution; there is no notice taken of factors affecting the aerial part of the plant. No attempt is made at an analysis of how the plant is affected in its various phases, and there is a complete lack of conception of the possibility of broader investigations on dynamic principles. There is a complete lack of correlation between the various physiological processes: germination, assimilation, root absorption, transpiration, respiration, permeability, etc. There is generally an absence of any observations relating to hereditary factors.

Under these circumstances it is no wonder that no simple principle has been brought out and no refined technique evolved in the case of water culture experiments, and that the whole method is more or less a matter of convention. Yet every piece of work shows clearly the complexity of the matter, and indicates more and more the necessity for the realisation of it, and the impossibility of settling definitely questions of root absorption before a number of these elementary difficulties resulting from complexity have been removed.

THE WATER CULTURE METHOD.

We may now consider in detail some of the more obvious matters which call for discussion in the water culture method.

1. *Hereditary factors.* In the majority of water culture experiments no account whatever is taken of the fact that differences may

appear between individuals of the same species on account of their different ancestry. In some recent work recognition of this fact has led to the use of pure lines of seed where inherited differences are reduced to a minimum. The interaction of hereditary factors and environmental factors has, however, never been dealt with in water cultures—that is, no attempt has been made to determine whether different strains of the same species will react differently to various environments.

Related to this question is that of the variability of different species as to the conditions which suit them best, and in general, their different modes of reaction to different environmental conditions. It has been far too common to draw conclusions as to the behaviour of plants in general from the behaviour of one or at most a few species, when there is no evidence or reasonable expectation that all species should behave in the same way.

2. *Germination conditions.* The germination of seeds for experimental work is a more serious matter than is generally assumed. The effect of preliminary soaking has been known for many years to have a great effect on the time required for germination, and on the vigour of the seedling produced. After effects may be caused by the germination conditions which are attributed to the later environment, and so completely false conclusions may be drawn. The effect of temperature on germination, the time of preliminary soaking in water, the medium in which germination takes place, as well as the different effects of these on different species, all require consideration.

3. *The technique of the bottle and cover.* The use of glass jars or wide mouthed bottles covered with paper or black paint to exclude light and growth of algæ is universal in water culture experiments, but there is no uniformity as to the size of the vessel. Thus in recent work of some American investigators (*e.g.* 9) jars of 250 c.c. were used to carry 6 plants, thus giving 42 c.c. of solution per plant. At the other extreme we have Wortmann (11) who recommends the use of vessels of 26,500 c.c. capacity for a single plant. Most investigators have used vessels intermediate in capacity between these two extremes. It is to be expected that the size of the vessel employed has influenced results with water cultures, principally owing to the different rates of change in the composition of the culture solution to which we shall refer below, but also possibly owing to other causes.

The cover of the vessel has generally taken the form of a cork

cut in various ways, between the sections of which the plant is supported in cotton or asbestos wool. The essential point is that the support given to the plant should be sufficient to keep it in place and at the same time not so great that the part of the plant surrounded by the cork is compressed so as to damage it or hinder its growth in width.

Means are also adopted to prevent the growth of fungi on the cork, as for instance by using new corks and immersing them before use in melted paraffin wax. Some workers use slabs of wax instead of cork. Growth of fungi on the experimental plant is prevented by keeping the upper surface of the nutrient solution out of contact with the cork or wool. Difficulties arising from this cause are probably greater in some cases than in others. Our experience is that a great deal too much has been made of them, and in many cases no trouble is likely to arise from this cause. Great care is usually taken to keep attached seeds out of the culture solution, but it is not clear whether this precaution is more necessary than many others which are completely neglected.

4. *The nutrient solution.* Examination of the literature reveals an enormous number of nutrient solutions which have been recommended by various investigators for general use in water culture experiments. These differ much among themselves both as to the total concentration of the solution and the relative proportions of the constituents. Attempts have been made to examine the effect of altering both these factors, with the most divergent results. Thus, some workers have found the concentration of the solution to have little or no effect on the growth of plants, others, on the other hand, have found the reverse and have stated a definite strength of solution as the optimum for plant growth. Naturally enough different observers find different optima. The explanation of this divergence in results is to be found in the failure to realise the complex interaction of a number of factors left unconsidered, as well as in the assumption that the culture solution is unaffected by the presence of the plant growing in it.

Similar considerations hold in regard to the influence of varying the relative proportions of the various constituents. For some time now the idea of *physiological balance* between the constituents of the nutrient solution has been gaining ground. The greatest rate of growth is supposed to result when the constituents are present in a certain definite ratio. It is again significant that the ratio found by various observers is different; it varies also for different concentrations and different species.

A view which has been held for many years is that the rate of growth depends on the hydrogen-ion concentration. Thus we find it asserted that it is necessary to keep the culture solution slightly acid and that chlorosis results if the solution becomes alkaline. In recent years there have been so many observations on the importance of hydrogen-ion concentration in physiological processes that there is every reason why the earlier observation of the importance of slight acidity should have been made.

The concentration of phosphate *dissolved* in the solution has also been held as of importance, but in spite of Benecke's attempt (1) at an investigation of the complex physical chemistry of water culture solutions it is not clear whether it is hydrogen-ion concentration, or the concentration and solubility of phosphate, iron, or other constituents which is of importance in the avoidance of chlorosis.

A possibility which has never been properly examined is that of changes with time in the composition of the dilute solution which constitutes the nutrient solution. It is possible that gradual changes of concentration of the various constituents may take place owing to such processes as hydrolysis and double decomposition, which will result in changes in hydrogen-ion concentration and phosphate concentration among others which may influence the rate of growth.

But this is probably a small matter in comparison with changes which take place in the nutrient solution as a result of plants growing in it. Causes working to alter the composition of the solution are absorption of constituents, and absorption of dissolved oxygen and excretion of carbon dioxide in respiration. There is also the possibility of the production of toxic substances in the solution either as the result of active excretion, as some American writers would hold, or as the result of decomposition of dead root tissue.

The absorption of constituents from the solution reduces the total concentration of the solution, but as the rate at which the constituents are absorbed depends not only on the concentration of the constituents outside, but also on the rate at which they are removed as such inside the plant, it is clear that the solution external to the roots must change in relative composition as well as in total concentration. Moreover, it appears that the constituent ions of any salt are absorbed independently of each other (6). As a result not only do the relative proportions of the constituents of the nutrient solutions alter with time, but also the hydrogen-ion

concentration changes as the solution becomes more acid or alkaline, and this has been known to be the case for fifty years.

The difficulty arising from changes in the nutrient solution as a result of absorption of solutes, can be obviated by continually renewing the culture solution, which is most satisfactorily done by passing a continuous stream of the solution through the culture vessel. Occasional renewal is the next best thing to do. It is significant that in all cases where the solution has been renewed at intervals increased growth of cultures has resulted, the increase being greater the more frequent the renewal (2, 7, 10) and greatest when the solution is constantly renewed by passing a continuous stream of solution through the vessel. The same treatment would also remove toxic excreta if such are indeed produced. In regard to changes produced in the concentration of dissolved gases in the solution owing to respiration, it is clear that decrease in the oxygen pressure outside may decrease the respiratory activity of the root, while the same effect may be produced by accumulation of carbon dioxide outside. Renewal of the solution will also tend to keep up the oxygen supply and to keep down the concentration of carbon dioxide.

The oxygen supply of the solution may also be kept up by bubbling air through the solution, and at the same time the removal of excess of carbon dioxide would be facilitated. But the expectation that aerated cultures would grow faster than non-aerated cultures under otherwise identical conditions has not always been realised. This diversity of result must again be due to the complexity of the processes involved.

Before leaving the question of the nutrient solution we may here make a note on the weighing out of salts for such solutions. Great care is often taken to use the purest salts obtainable, which are then weighed out with the greatest exactness. Now all our experience goes to show that differences of a few per cent in the total concentration and in the relative proportions of the different nutrients produce no difference in growth which is measurable by the water culture method. To obtain such accuracy in the initial constitution is simply an unnecessary waste of time when the low degree of accuracy obtainable by the water culture method is considered, and when it is remembered that the constitution of the solution is constantly changing, and that the rate of change will depend, among other things, upon the absorptive activity of the root system, which is unlikely to be the same in all the cultures in one experiment.

5. *Climatic factors.* Those factors influencing the growth of the plant which act through the aerial part, and which we may conveniently group under the term 'climatic factors,' are of great importance in determining the activity of the plant, yet they are never taken account of in water culture experiments. They are principally temperature, light and carbon dioxide supply, on which assimilation is highly dependent. The effect of all these factors on influencing assimilation and consequently growth is well known, and their complex mode of interaction has been emphasized. As a simple example of how neglect of them may vitiate conclusions drawn from water culture experiments we may refer to the different results which might be obtained in winter and summer. Plants growing under two different sets of conditions at the higher temperatures and illuminations of summer might show different rates of growth on account of those differences in environment, whereas at the lower temperature and under the diminished illumination of winter, no differences in growth in the two cases might result, although those differences in environment maintained in the summer experiments were maintained also in the winter ones. This would be due simply to the fact that the temperature and light were limiting growth during the winter period, and if it were concluded that the differences in the other factors of environment produced no effect the conclusion would be false.

As well as the factors of temperature and light, the carbon dioxide supply must be taken into consideration. This is not nearly so constant a factor as is generally supposed; it makes a considerable difference whether the air is stagnant or kept in motion. Movement in the air, by breaking down the diffusion shells which are otherwise produced over the stomata, is equivalent to an increase in the concentration of the carbon dioxide.

A factor depending on these is that of spacing. If plants are growing closely together there may be competition between them for temperature, light and carbon dioxide, the term competition being used, of course, in its biological sense. Thus in the work of many experimenters in America, a number of plants are grown together in one culture jar. Recent work at Rothamsted has shown that when two plants are grown together in one culture vessel, a decrease in growth of the individual plant results, which is to be ascribed to competition between the aerial parts (3).

6. *Increase in Dry Weight as a Criterion of the Activity of the Plant.* As we have emphasized in the general introduction to this paper, the measurement of dry weight affords no indication of the

general activities of the plant. Flower and fruit formation, or even final yield of vegetative material, do not necessarily follow the total increase in growth after an arbitrary time. Such statical methods have caused the drawing of wrong conclusions in other branches of plant physiology.

On the other hand the measurement of dry weight is much to be preferred to the measurement of fresh weight, which is subjected to all the drawbacks of the measurement of dry weight and to this as well, that the fresh weight will vary during the day according to the rate of transpiration.

7. *The accuracy of the results obtained in the Water Culture Method.* The differences which may result on account of hereditary factors and on account of unsuspected differences in environmental factors during germination and later stages of the life of the plant, make it necessary to determine the degree of accuracy of the results obtained. The vast majority of the results obtained by the water culture method are absolutely valueless as no probable errors of experiment are calculated, and the results are given in such a form that it is impossible to calculate them. Often differences regarded by the investigators themselves as significant are obviously not so.

We would emphasize again, as formerly, the necessity of calculating the probable error of experiment when water cultures are used, as only in this way can the significance of obtained differences be appreciated.

THE PRESENT EXPERIMENTS.

The question with which we deal particularly in this paper is that of aeration of the culture solution, a question which has received some attention in recent years. Thus Hall, Brenchley and Underwood (5) record greatly increased growth as measured by dry weight as a result of continuous aeration of the solution. Their conclusions were obtained with Barley and Lupin grown in sets of four. Their results are shown in the accompanying table.

			Aerated continuously.	Aerated once a day.	Not aerated.
Barley	2.122		1.314
Lupin	1.53	0.72	0.83

The increase is striking and greater than differences due to experimental error, as can be calculated from the data of the weights of individual plants recorded in the paper.

It is now well established that, in all cases so far investigated at any rate, changing the culture solution increases the rate of growth (2, 7, 10). It is, therefore, interesting to determine whether the factor reducing the growth-rate in non-aerated cultures is removed when the solutions are constantly renewed, or whether aeration still increases the rate of growth even if the solutions are frequently renewed. It is also of importance to determine whether alteration in other factors produces any difference in the effect of aeration, and for this reason different culture solutions were used in different experiments. Attempts were made to control the temperature and light-intensity under which the cultures were grown, but these had to be abandoned owing to a number of difficulties which it was found impossible to surmount under present conditions.

These experiments were already in progress when there reached us a preliminary statement of the results of experiments on the aeration of water cultures grown in Livingston's laboratory at Baltimore (4). In these experiments ordinary aeration of water cultures of Buckwheat produced no effect at all on the rate of growth, while bubbling oxygen or nitrogen through the cultures also produced no result. Carbon dioxide on the other hand caused the death of the plant.

These divergent results of the workers at Rothamsted and Baltimore make it all the more desirable that results obtained with more numerous individual cultures in which the significance of the observed differences is measured should be recorded. In the experiments here described we have worked with sets of ten plants under the same conditions, and the probable error of results has been calculated.

The bottles used were of one litre capacity, and the usual procedure in regard to the bottle and cork was followed. One plant was used for each vessel. Aeration was produced by means of an air compressor to which was connected "composition" tubing provided with a number of branch tubes. These were joined by rubber tubing to glass tubes which passed through the corks of the bottles to the bottom of the culture vessels. There was no difficulty in aerating thirty cultures at once.

The nutrient solutions were made up from ordinary "pure" salts and London tap water. The solutions were renewed at frequent intervals except in some cases in which no renewal took place. Three different solutions were used which will be described under

the results of the experiments themselves. The experimental plant chiefly used was Barley, but supplementary experiments were also made with Buckwheat and Balsam. The seeds were soaked in water for 24 hours at laboratory temperature (about 20° C), and were germinated in moist Sphagnum. The cultures were harvested in all cases 50 days after sowing in Sphagnum. Each culture occupied a floor space of about 15 cm. × 12 cm. = 180 sq. cm.

EXPERIMENTAL RESULTS.

Barley.

The seed of Barley was of a pure line, and was kindly sent by Professor Biffen. The seed was sent in the ear, and in each series the seed used was all obtained from a single ear. In this way errors due to inherited differences should be reduced to a minimum. Ten cultures were aerated, and the same number non-aerated.

Series 1.

In the first series Crone's solution composed as follows was used:

Potassium nitrate	1.00 gm.
Magnesium sulphate (cryst.)	0.25 „
Calcium sulphate	0.25 „
Ferric phosphate	0.25 „
Tap water	1000.00 cc.

Seeds sown in Sphagnum, May 2.

Seedlings transferred to culture vessels, May 10.

Solutions changed, May 23, 30, June 6, 11, 16.

Cultures harvested, June 21.

An attempt was made to follow the rate of growth by counting the number of leaves at intervals. The results are shown in the following table:

Days after sowing	Average number of leaves per plant	
	Aerated	Non-aerated
8	2.0	2.0
17	3.0	3.0
24	4.0	4.0
29	5.4	4.7
33	6.4	5.6
35	7.7	6.0
36	9.1	6.6
41	12.0	8.9
43	14.5	9.6
45	15.8	10.8
48	19.4	12.0
50	20.3	12.7

After 50 days from sowing the aerated cultures thus show a markedly greater leaf development, namely an increase of

Influence of Aeration of Nutrient Solution. 191

60 per cent. Up to the twenty-fourth day after sowing no difference was obvious, but by the twenty-ninth day the difference began to appear and steadily increases. In other words the difference begins to appear with the fifth leaf.

A similar improvement was observed in the dry weight at the end of the period. The figures obtained are shown, together with probable error in each case.

				Aerated.	Non-aerated.
Shoot	0.943	0.585
Root	0.217	0.128
Total	1.160 ± 0.035	0.713 ± 0.041

This gives an increase in dry weight resulting from aeration of 63 per cent., many times greater than the probable error.

Series 2.

The food solution used in this series consisted in a modification of Crone's solution, namely

Potassium nitrate	1.00 gm.
Magnesium sulphate (cryst.)	0.25 „
Calcium sulphate	0.25 „
Potassium monohydrogen phosphate	0.25 „
Ferric chloride	0.10 „
Tap water	1000.0 cc.

This series ran contemporaneously with Series 1, but the solutions were left unchanged through the whole growth period. The culture jars were however filled up with water towards the end of the experiment, namely on June 16, the 45th day after sowing.

Similar results were obtained as in the case of Series 1, both in regard to leaf development and dry weight after 50 days. The numbers actually obtained were as follows :

Days after sowing	Number of Leaves	
	Aerated	Non-aerated
8	2.0	2.0
17	3.0	3.0
24	4.0	4.0
29	5.3	4.9
33	6.1	5.6
35	7.6	6.1
37	8.3	6.7
41	11.3	8.5
43	13.3	9.6
45	14.8	10.9
48	17.1	12.0
50	18.3	12.7

The differences in the rates of leaf development are thus about the same as in Series 1. After 50 days the increase is 44 per cent. The differences in dry weight after that time are similar.

	Aerated.	Non-aerated.
Shoot	0.982	0.623
Root	0.204	0.150
Whole Plant	1.186 \pm 0.083	0.773 \pm 0.041

The increase in growth in the aerated cultures is thus 53 per cent.

Series 3.

The solution used in this series was essentially that recommended by Pfeffer. It was constituted as follows:

Calcium nitrate (cryst.)	1.00 gm.
Magnesium sulphate (cryst.)	0.25 „
Potassium nitrate	0.25 „
Potassium chloride	0.06 „
Potassium dihydrogen phosphate	0.25 „
Ferric chloride	0.04 „
Tap water	1000.00 cc.

Seeds sown in Sphagnum, June 20.

Seedlings transferred to solution, June 26.

Solutions changed, July 6, 14, 20, 25, 28, 31, August 3, 6.

Cultures harvested, August 9.

The leaf development was as follows:

Days after sowing	Number of Leaves	
	Aerated	Non-aerated
10	2.0	2.0
15	3.0	3.0
19	4.0	4.0
23	5.8	4.8
26	7.0	7.0
30	11.7	9.9
34	15.4	14.1
43	25.5	21.2
47	29.6	24.1

The difference in the rate of leaf development in aerated and non-aerated cultures was thus considerably less than in the case of Series 1 and 2, the increase resulting from aeration being only 23 per cent. after 47 days. Whether this is to be correlated with the differences in the culture solution or with unmeasured factors

in the aerial environment it is not possible to say. The atmospheric conditions seemed much the same in the three series of experiments, nor was there much difference in the total period of illumination. Nevertheless in the absence of exact measurement it is impossible to draw any conclusions in the matter. It will also be observed that the total leaf development is considerably greater in the third series as compared with the first and second. This improvement is also shown in the dry weight results. We give the results of individual plants in order to show the sort of differences obtained between different individuals when steps are taken to reduce inherited differences to a minimum.

Aerated.

Plant	Dry Weight in grams		
	Shoot	Root	Whole Plant
1	1.553	0.722	2.275
2	1.328	0.600	1.928
3	1.393	0.701	2.094
4	1.250	0.624	1.874
5	1.503	0.624	2.127
6	1.584	0.733	2.317
7	1.434	0.629	2.063
8	1.532	0.619	2.151
9	1.480	0.442	1.920
10	1.482	0.858	2.340
Mean		...	2.109 \pm 0.025

Non-aerated.

Plant	Dry Weight in grams		
	Shoot	Root	Whole Plant
1	1.306	0.446	1.752
2	1.135	0.549	1.684
3	0.918	0.369	1.287
4	1.006	0.320	1.326
5	1.106	0.334	1.440
6	1.316	0.458	1.774
7	1.471	0.487	1.958
8	1.601	0.501	2.102
9	0.909	0.308	1.217
10	1.837	0.582	2.419
Mean		...	1.696 \pm 0.083

This shows an increase of dry weight of the aerated cultures over the non-aerated of 24 per cent., after 50 days from sowing. This is practically the same as the increase in leaf development. It will be observed that the probable error of experiment in the case of the dry weight of the aerated cultures is 1.2%, and in the case of the non-aerated 4.7%. The recorded difference in dry weight

between the two sets of cultures is thus several times greater than the probable error, and there can be no doubt of its significance.

The results thus agree with those obtained by Hall, Brenchley and Underwood with Barley.

Buckwheat.

Series 4.

The recent experiments of Free (4) at Baltimore failed to show any improvement in the growth of water cultures of Buckwheat as a result of aeration.

The seed used in this series was obtained from Messrs. Carter. The culture solution was composed as follows :

Calcium nitrate (cryst.)	1.00 gm.
Magnesium sulphate (cryst.)	0.25 „
Potassium nitrate	0.25 „
Sodium chloride	0.06 „
Potassium dihydrogen phosphate	0.25 „
Ferric chloride	0.04 „
Tap water	1000.00 cc.

Seeds sown in Sphagnum, July 5.

Seedlings transferred to solutions, July 11.

Solutions changed, July 21, August 13, 22.

Cultures harvested, August 24.

The leaf development was not followed in this series. The dry weights obtained after 50 days from the date of sowing are given below. They are given in full as they are instructive in comparison with the numbers obtained in the previous series as showing the great effect of inherited factors in water culture experiments. The buckwheat seed was not a pure line, and in consequence variations due to inherited factors are much more prominent.

Aerated.

Plant	Dry Weight in grams		
	Shoot	Root	Whole Plant
1	1.977	0.190	2.167
2	0.680	0.078	0.758
3	1.256	0.161	1.417
4	1.362	0.128	1.490
5	1.763	0.157	1.920
6	1.507	0.163	1.670
7	0.271	0.044	0.315
8	1.090	0.202	1.292
9	0.943	0.090	1.033
10	1.775	0.146	1.921
Mean	1.398 \pm 0.120

Non-aerated.

Plant	Dry Weight in grams		
	Shoot	Root	Whole Plant
1	1.363	0.220	1.583
2	1.444	0.230	1.674
3	1.092	0.105	1.197
4	1.534	0.190	1.724
5	1.935	0.276	2.211
6	1.592	0.178	1.770
7	1.444	0.124	1.568
8	1.350	0.191	1.541
9	1.235	0.178	1.413
10	0.610	0.081	0.691
Mean	1.537 ± 0.084

When the probable error is taken into consideration it must be concluded that there is no significant difference between the dry weight of the aerated and non-aerated cultures. But the experiment does not measure differences less than 10 per cent., as a recorded difference of this value has no real significance.

This result agrees with that obtained by Free at Baltimore. It does not however indicate that under *all* circumstances it would be impossible to obtain improved growth of buckwheat in water culture by aeration.

Balsam.

Series 5.

The seed of Balsam used was "Sutton's Improved Rose-flowered," and was kindly sent by Messrs. Sutton. The seeds varied considerably in size, and so they were graded before germination, those used having a weight between 0.01 and 0.02 gm.

Owing to the small quantity of seed available only 10 cultures in all were grown, 5 aerated and 5 non-aerated. The dry weight was taken 50 days after sowing as before. The culture solution was the same as that used in Series 3.

Seeds sown in Sphagnum, July 11.

Seeds transferred to solutions, July 19.

Solutions changed, July 28, August 14, 22.

Cultures harvested, August 30.

The dry weights were obtained as follows :

	Aerated.	Non-aerated.
Shoot	1.9985	1.309
Root	0.5795	0.452
Whole Plant	2.578 ± 0.201	1.762 ± 0.178

Here the recorded difference in dry weights is considerable (46 per cent.). The probable errors are however large owing to the small number of plants and individual differences between the plants. Nevertheless the difference between the dry weights of the aerated and non-aerated cultures is between two and three times as great as the sum of the probable errors, and it seems legitimate to regard this difference as having a real significance.

CONCLUDING REMARKS.

In the foregoing pages we have emphasized the complexity of problems of water cultures, and have endeavoured to illustrate it especially in relation to aeration. Thus we have obtained similar results to those of Hall, Brenchley and Underwood in relation to the increase in growth of barley resulting from aeration. On the other hand with buckwheat we have obtained a result similar to that of Free who found no benefit result from aeration.

It does not follow however that the same result would be obtained under all conditions of experiment. It is very likely that there are conditions in which aeration would produce no effect on the rate of growth of barley, or which would bring about an increase in the rate of growth of buckwheat. . .

We have also shown how different conditions bring about different increases in the rate of growth as a result of aeration (contrast Series 3 with Series 1 and 2).

There is at present no explanation why under the conditions of our experiments aeration should increase the rate of growth of some species and not of others. We do not know whether it is to be correlated with oxygen supply, removal of carbon dioxide, removal of diffusion gradients, or change in solubility conditions or other causes. There seems no doubt however that water culture conditions react in a different manner on different species.

It must be emphasized that a great deal of work must be done on the physical chemistry of water culture solutions before work can be done with necessary definiteness. Particularly investigation must be extended on dynamical principles as is clearly indicated from the investigations of Pantanelli (6).

Further it is obviously desirable to work towards getting some more explicit principle which will take into account the co-operation of the various activities of the plant. It seems clear that at present neither the law of the minimum nor the principle of limiting factors as employed by Blackman in regard to the sub-aerial part

of the plant are expressions of our present knowledge of the life of the plant as a whole.

The detachment between plant physiology and agriculture might be removed if a working principle in regard to the physiological relations of plant processes were evolved which embodied the activities of both sub-aerial and subterranean parts of the plant. This is highly desirable, but it must be admitted that it seems far away at present.

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THE ORIGIN AND DEVELOPMENT OF THE COMPOSITÆ.

BY JAMES SMALL, M.Sc. (LOND.), PH.C.

CHAPTER II.

THE POLLEN-PRESENTATION MECHANISM.

IN the Compositæ the mechanism involved in the presentation of pollen to the insect visitor includes both styles and stamens. These essential organs are discussed, therefore, in the present chapter, but for convenience the variation in structure of styles and stamens is described and discussed in separate sections, while their functions in the pollen-presenting mechanism is considered partly in section D of the present chapter and partly in Chapter III which deals with the irritability of the mechanism.

A. HISTORY.

The history of our acquaintance with the pollen-presentation mechanism may be divided into two distinct sub-sections, the first dealing with the development of our knowledge of the details of the essential organs and the second dealing similarly with the process of pollination.

Structure.

Pre-Cassinian Period.

Before the classic investigations of Malpighi and Grew there is no mention of either styles or stamens in the descriptions of Compositæ. Nehemiah Grew in his first book (29, 1671) describes the floret of the Compositæ as an "epitome of a flower." The anther tube and style are described and figured, and various secondary uses of these parts are given but the "Primary and Private Life I now determine not." In 1675 Malpighi (46) published good figures and descriptions of the styles and stamens in *Cichorium*, and in the following year Grew's fourth book appeared with numerous excellent figures and descriptions of the styles and stamens of several Composites. The Compositæ also furnish the basis of his chapter on "The Use of the Attire," a rather crude account of self-pollination in which both the style or "blade" and the stamens or "sheath" are considered to be male organs, the "Thecæ of the Sheath" being described as the "Vegetable Sperme."

Origin and Development of the Compositæ. 199

The first systematist to mention the characters of the styles and stamens as described by Malpighi and Grew was Vaillantus (70). Boerhaave in his first *Index* (7) did not give the distinguishing characteristics of his sub-divisions but did so in his *Index alter* (7). The syngenesious anthers and bifid stigma were now established as characteristic features of the family. Pontedera (56), distinguishes *Valeriana* by the stamens not being in a tube and also distinguishes other syngenesious forms, such as *Asclepias* and *Cucurbitacæ* by their non-capitulate form, while Linné (43) uses the character as the primary one in his *Syngenesia* of the *Sexual System*. No further details of structure are given by subsequent authors (6, 25, 35, 59) up to the beginning of the Cassinian period.

Cassinian Period.

Cassini (13) described the styles and stamens in great detail and used these details in his classification. Don subsequently used the basal appendages of the stamens in his classification of the *Cichorieæ* (20) and also records (21) abnormalities in several species of *Zinnia* with 4-10 stamens, 3-10 stigmas and 2-5 embryos. The styles and stamens were also used by Link (42), but his work is really of the pre-Cassinian type. Lessing (41) described the general staminal characters in great detail but used chiefly the stylar characters in his *Synopsis*, where he neglects the distinctive features of the stamens except in three tribes. De Candolle (17) used stylar characters for the tribes, but staminal characters for some of his sub-tribes, which were practically those of Cassini. The structure of the style was studied in great detail by Hildebrand (32) and by Chamberlain (14). The rudimentary stamens noted by the former in abnormal specimens were noted also by Cassini (13), Masters (48) and others.

Bentham (4,5) realised the importance of the details of both styles and stamens and used both for tribal and generic characters. Le Maout and Decaisne (40), Asa Gray (28) and other systematists agreed with Bentham and Cassini, while Baillon (2), using styles chiefly for his tribes, divided several of them into "sous-séries" on the anthers. The importance of the anther appendages in the biological efficiency of the pollen-presentation mechanism was emphasised by the present writer (61).

The development (9) and the vascular supply (30, 63, 67) of the styles and of the stamens (63, 67) and the structure of the pollen grain (3, 62, 71) have been described by various authors. Martin (47) considered that the tissue of the contiguous anthers

was in organic connection, while Coulter (15) and Merrell (49) report no such fusion. Observations by the writer prove that in some cases, at least, no organic connection exists, but the possibility of fusion in some species is not excluded and further research is in progress on this point.

Pollination.

Camerarius (10) was the first to conduct experiments in artificial pollination and to study pollination in the Compositæ. Miller (50) was the first to observe pollination by insects, but our knowledge of pollination in the Compositæ is founded on the later work of Kölreuter (38 and see Chap. III) and Sprengel (64). The latter noted the advantages of the capitulum and other characters in the Compositæ, and dealt with *Tussilago*, *Silybum*, *Centaurea*, etc. The next advance was about 40 years later when Brongniart (8) started a controversy on the function of the collecting hairs of the Campanulaceæ which led to a re-examination of the Compositæ for comparison by Hassall (31), Wilson (72), Todd (65) and Trelease (68). About this period also the study of pollination progressed rapidly under the stimulus of Müller's work (51), and the modern aspect of the subject together with the literature may be found in Knuth's compilation (36) based on Müller's contributions. The biology of *Helianthus annuus* and other Compositæ was the subject of several papers (12, 22, 27, 53), and Darwin (16) notes the sterile, pollen-presenting style which occurs in the male florets.

A valuable contribution was made by Juel (34) who distinguishes four chief types of pollen-presentation: (1) pollen presented on the tip of anther-tube, (2) pollen presented on the outer surface of the style and stigma, (3) pollen presented on the inner surface of the corolla lobes, (4) pollen presented directly from the anthers (anemophilous). Types 3 and 4 are comparatively rare, while type 1 is divided into two subsidiary types on the length of the male stage of anthesis, and type 2 has four forms, the *Gaillardia* type, the *Eupatorium* type, the Cichoriæ type and the *Arctotis* type, which differ in slight details.

The distribution of the sexes in the capitulum formed the basis of Linné's divisions of the Syngenesia, and this part of the subject has been studied exhaustively by Uexküll-Gyllenband (69) whose conclusions may be summarised as in Fig. 4 from her "Generalschema für die phylogenetische Blütentwicklung." In

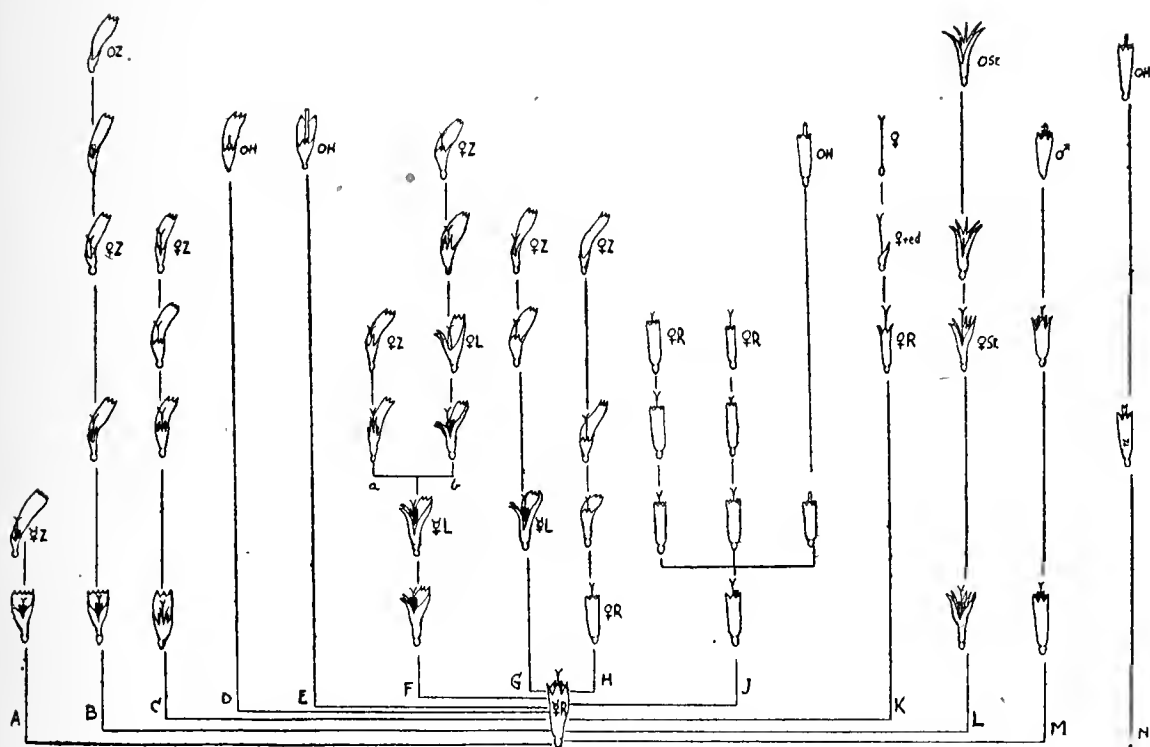


FIG. 4. Phylogeny of Flower forms (after Uexküll-Gyllenband).

A—*Hieracium barbatum*—Cichoriææ. B—*Anthemis Cotula*—Anthemideæ. C—*Psiadia glutinosa*—Astereæ. D—*Heterothalamus brunioides*—Astereæ. E—*Xeranthemum annuum*—Cynareæ. F—*Gerbera* { a. *Wrightii* } Mutisieæ. G—*Anandria tomentosa*—Mutisieæ. H—*Erigeron alpinus*—Astereæ. J—*Leontopodium alpinum*—Inuleæ. K—*Haastia Sinclairi*—Astereæ. L—*Centaurea Jacea*—Cynareæ. M—*Leontopodium alpinum*—Inuleæ. N—*Petasites albus*—Senecioneæ.

♂ R=hermaphrodite tubular floret. ♀ Z=hermaphrodite ligulate floret. ♀ L=hermaphrodite bilabiate floret. OZ=neutral ligulate floret. OH=neutral honey floret. OSt=neutral ray floret. ♀ Z=female ligulate floret. ♀ L=female bilabiate floret. ♀ R=female tubular floret. ♀ St=female ray floret. ♀ red=female floret with reduced corolla. ♀ =female floret with no corolla. ♂ =male floret. Figures unmarked are intermediate forms occurring in the same capitula.

addition there have been many other studies of sex-distribution in the capitulum, such as 26, 33, 57 and 58.

Parthenogenesis and apogamy in the family have been studied by Murbeck (52), Ostenfeld (54-55), Schkorbatow (60) and others. The flowering period, which is usually autumnal in the north temperature regions, is the subject of notes by Bailey (1) and Lebard (39). Anemophily in the family is discussed at length by Delpino (18-19) and ornithophily in the Compositæ, Campanulaceæ and allied families is noted by Fries (24) and Trelease (68). The secretion of an intoxicating nectar in abnormal cases by *Centaurea Scabiosa*, *Carduus nutans*, *C. lanceolatus* and other species has been observed by Lloyd Williams (44).

The action of pollen grains mixed in various proportions from differently coloured capitula is the subject of a note by Lowe (45),

and the artificial germination of the pollen grains still presents difficulty. Ostenfeld (55) stated that "no one has been able to germinate the pollen grains of any Composite in artificial culture," but two years later Tokugawa (66) germinated the pollen of various Composites on the stigmas of plants belonging to other families, for instance the pollen of *Taraxacum albiflorus* and *Matricaria Chamomilla* germinated on the stigma of *Yucca gloriosa* and that of *Dahlia variabilis* on the stigma of *Abelmoschus Manihot* and *Hibiscus mutabilis*.

B. STYLES OF THE COMPOSITÆ.

The general type in the family is a slender style with two branches which are stigmatic on the inner surfaces. The style and style branches may be hairy or papillose or glabrous externally; style branches have appendages of various types, and the hairs of these appendages are arranged so that they either push or pull the pollen out of the anther tube; the stigmatic papillæ are conspicuous and arranged along the margins of the inner surfaces of the style branches, or they are less conspicuous and arranged either marginally or all over the inner surfaces. The vascular supply of the style is composed usually of two strands given off from a region of anastomoses at the top of the ovary; where the style is thick and practically undivided there are sometimes four vascular strands, as in *Arctotis* and *Tussilago* (63). The style in some genera is sensitive to touch (see Chap. III).

Types of Style.

The numerous variations can be reduced to fourteen types which will now be described.

Type I. Style branches slender, subterete or slightly flattened, papillose or glabrous externally; stigmatic papillæ all over inner surface from base to apex (Fig. 5, I).

Type II. Style branches elongated or short, subterete below, thickened and club-shaped or flattened and obtuse above, papillose or glabrous externally; stigmatic papillæ marginal and inconspicuous, confined to the lower part of the style branches. (Fig. 5, II and IIa). The upper sterile portions of the style branches are appendages.

Type III. Style branches elongated, subterete, subulate, more or less acute at the apex, hairy externally; stigmatic papillæ inconspicuous, extending in the centre of the inner surfaces of the style branches usually from the base to near the apex, but sometimes by

abortion of the upper stigmatic papillæ confined to the lower part of the style branches (Fig. 5, III).

Type IV. Style branches elongated or short, flattened, truncate at the apex, hairy or papillose externally; each branch ending in a brush of hairs considerably thicker and longer than those which clothe the outer surface; stigmatic papillæ in more or less conspicuous marginal lines, extending from the base to near the apex of the branches (Fig. 5, IV).

Type V. Style branches truncate and penicillate as in IV but with the inner surfaces permanently together (*i.e.*, the style branches do not diverge); stigmatic papillæ absent; very similar to IV before divergence of the style branches has taken place; characteristic sterile pollen-presenter in male florets (Fig. 5, V).

Type VI. Upper part of the style more or less swollen and hairy; style branches very short; stigmatic papillæ all over the inner surfaces of the style branches (Fig. 5, VI).

Type VII. Style branches elongated or short, flattened, each ending in a triangular appendage covered externally and internally with hairs which are usually longer near the base of the appendage; stigmatic papillæ in conspicuous marginal lines extending from the base of the style branches to the base of the appendages (Fig. 5, VII and VIIa).

Type VIII. Style branches as in VII, but appendages conical and more elongated (Fig. 5, VIII).

Type VIIIa. Style branches as in VII, but stigmatic region short in comparison with the long, slender appendages (Fig. 5, VIIIa).

Type IX. Upper part of style more or less swollen and hairy externally; style branches very short or practically absent; stigmatic papillæ all over inner surfaces (Fig. 5, IX).

Type X. Style branches very hairy externally, especially on the outside of the stigmatic part which ends in a collar of long hairs; style divided to the collar or more deeply; stigmatic papillæ all over inner surfaces of style branches above the collar (Fig. 5, X and Xa).

Type XI. Style as in X, but with the style branches remaining practically closed; stigmatic papillæ as in X but exposed only by a slight divergence of the style branches (Fig. 5, XI).

Type XII. Style branches elongated or short, flattened, hairy externally either from base to apex or only at apex, apex rounded; stigmatic papillæ inconspicuous, in two marginal lines which may

be narrow or so broad that only a narrow non-papillate region in the centre is left; very similar to IV, but distinguished by the rounded apex and frequently by the broader stigmatic lines (Fig. 5, XII and XIIa).

Type XIII. Style undivided, hairy externally, club-shaped, stigmatic papillæ absent; very similar to XII before the divergence of the style branches has taken place, characteristic sterile pollen-presenter of some male florets (Fig. 5, XIII).

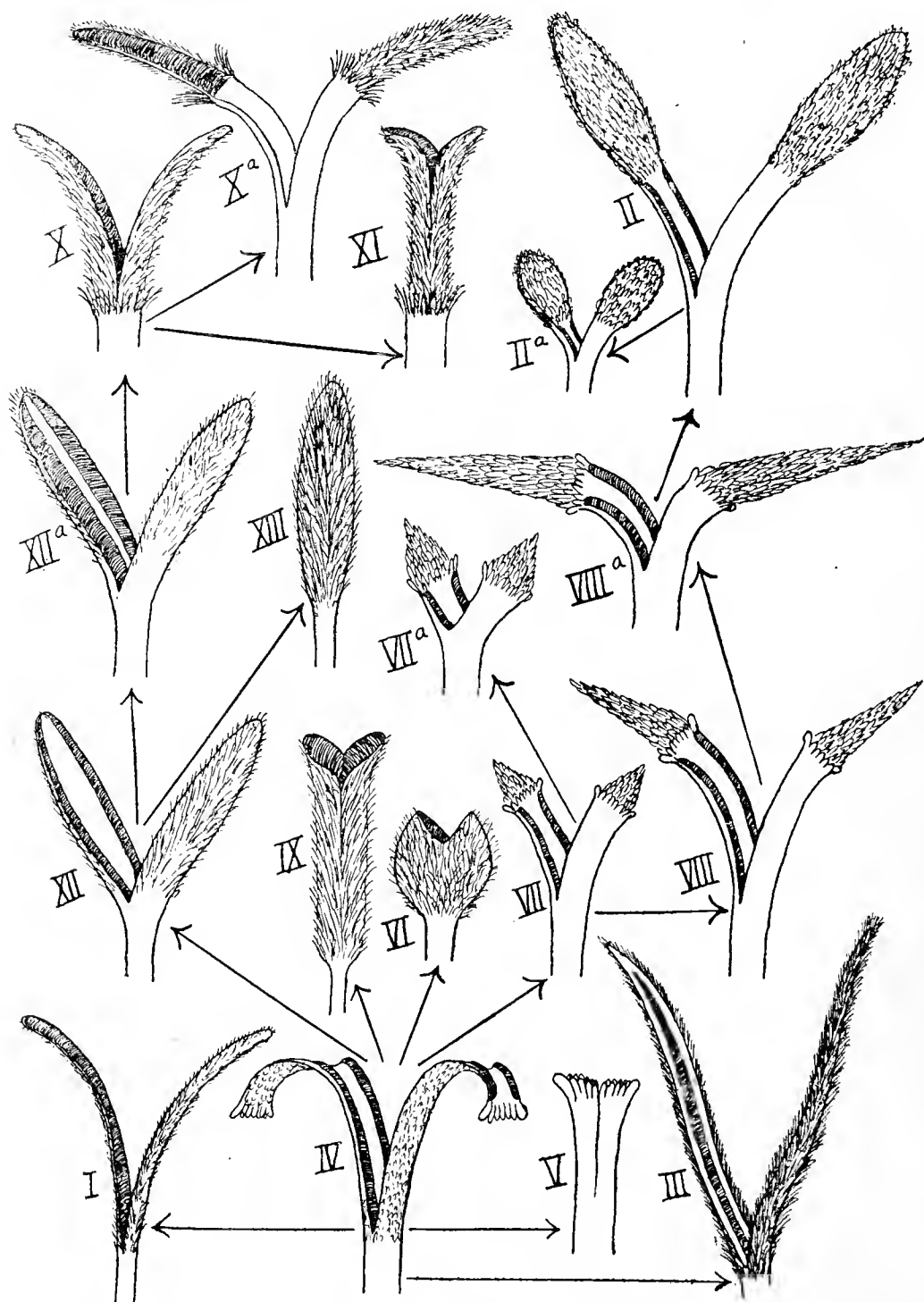


FIG. 5. Style forms in the Compositæ. The arrows indicate the lines of evolution.

Analysis of the Styles of Compositæ.

Analysis of the occurrence in the family of these various types of styles shows that type IV is the only one common to all except the more specialised tribes; it is, indeed, the typical style for the family, and the others are modifications of it. It will be obvious from the arrangement in Fig. 5 how type I has arisen from type IV by the disappearance of the truncate, penicillate apex and the elongation of the style branches. Type IV has also given rise to type V by the abortion of the stigmatic papillæ and the non-divergence of the style branches; to type III by the development of an awl-shaped apex and the transference of the stigmatic papillæ to the centre of the style branches; to types VI and IX by the spreading of the stigmatic papillæ and the partial fusion of the style branches; to type XII by the rounding of the tips of the style branches and the slight spreading of the stigmatic lines; and to type VII by the slight elongation of the hairy, apical part of the style branches (all stages of this change can be observed).

Types VII and XII then give two lines of development. Type VII by the shortening of the style branches gives VIIa; by the elongation of the apical appendages it gives VIII and then VIIIa, which last by a flattening and thickening of the appendages gives II. Type II by a shortening of the style branches gives IIa. Type XII by the abortion of the stigmatic papillæ and fusion of the style branches gives XIII; by the spreading of the stigmatic lines it gives XIIa. Type XIIa by the further spreading of the stigmatic papillæ over the whole of the inner surface of the style branches and the development of a collar of long hairs gives type X, which by splitting more deeply gives Xa, and by partial fusion of the style branches gives type XI. Types V and XIII with no stigmatic papillæ occur in the male disc florets of some genera.

Having elucidated the probable evolution of the various style forms we can now analyse the composition of the tribes and sub-tribes of the family as regards their styles. In Table VI Bentham's classification is followed for reasons which will be obvious from the summary of the history of classification given in Chapter I, Section A. Engler's system of terminology is used, however, for the sake of uniformity. Various new genera are included, but the list is not quite complete.

The numbers are obtained by counting 1 for each genus in the sub-tribe which shows a given type of style. If the genus shows two types of style then it is counted $\frac{1}{2}$ in each of the proper

TABLE VI. *Analysis of Style-forms in the Compositæ.*

TRIBES AND SUB-TRIBES			TYPES.														
			1	2	3	4	5	6	7	8	8a	9	10	11	12	13	
Vernonieae.																	
Vernoniinae			30												
Lychnophorinae			13												
		Total			43												
Eupatorieae.																	
Piqueriinae		8													
Ageratinae		23													
Adenostylinae		10													
		Total		41													
Astereae.																	
Homochrominae			1 $\frac{1}{2}$	1 $\frac{1}{2}$			5 $\frac{1}{2}$	13	6 $\frac{1}{2}$				1	1 $\frac{1}{2}$	
Grangeinae			1 $\frac{1}{2}$				3 $\frac{1}{2}$	1	1 $\frac{1}{2}$					1 $\frac{1}{2}$	
Bellidinae				2			5 $\frac{1}{2}$	2 $\frac{1}{2}$						1 $\frac{1}{2}$	
Heterochrominae				3 $\frac{1}{2}$			13	21 $\frac{1}{2}$	4 $\frac{1}{2}$					1	
Conyzinae							6	4 $\frac{1}{2}$	1 $\frac{1}{2}$					1	
Baccharidinae							1 $\frac{1}{2}$	1 $\frac{1}{2}$						1	
		Total			2	6			34	44	12				1	6 $\frac{1}{2}$	
Inuleae.																	
Tarchonanthisae					1								1	1	
Pluchinae				6									5 $\frac{1}{2}$	6	
Filagininae				4									6 $\frac{1}{2}$	1	
Gnaphaliinae				32 $\frac{1}{2}$	1								8	6 $\frac{1}{2}$	
Angianthisae				11									1		
Relbaniinae				12									2		
Athrixiinae				2 $\frac{1}{2}$									4	1 $\frac{1}{2}$	
Inulinae				2 $\frac{1}{2}$									21 $\frac{1}{2}$		
Bupththalmiinae				6 $\frac{1}{2}$									11	1	
		Total			1 $\frac{1}{2}$	77	2								60 $\frac{1}{2}$	16	
Heliantheae.																	
Lagasceinae			1												
Milleriinae							1	1 $\frac{1}{2}$						9 $\frac{1}{2}$	
Melampodiinae														22	
Ambrosiinae					7									2	
Petrobiinae					2										
Zinniinae			1	1			1	3						1	
Verbesiniinae			2 $\frac{1}{2}$	13 $\frac{1}{2}$			25 $\frac{1}{2}$	16					1 $\frac{1}{2}$		
Coreopsidinae			1	1 $\frac{1}{2}$			7	8 $\frac{1}{2}$						1	
Galinsoginae				2 $\frac{1}{2}$			4	2 $\frac{1}{2}$							
Madiinae			1 $\frac{1}{2}$				3	2						1 $\frac{1}{2}$	
		Total			7	17 $\frac{1}{2}$	9		44 $\frac{1}{2}$	29 $\frac{1}{2}$					1 $\frac{1}{2}$	36	
Helenieae.																	
Jaumeinae			1	2			3	1							
Baeriinae				10	1		15 $\frac{1}{2}$	7					1 $\frac{1}{2}$		
Flaveriinae				3											
Tagetinae				6			4 $\frac{1}{2}$	3 $\frac{1}{2}$							
Heleniinae				5 $\frac{1}{2}$			1 $\frac{1}{2}$	1 $\frac{1}{2}$							
		Total			1	26 $\frac{1}{2}$	1		24 $\frac{1}{2}$	12					1 $\frac{1}{2}$		
Anthemideae.																	
Anthemidinae				14	1										
Chrysanthemidinae				34	1										
		Total				48	2										
Senecioneae.																	
Liabinae			7												
Tussilaginatae		4					1							2	
Senecioninae				19			5 $\frac{1}{2}$	5 $\frac{1}{2}$	1				21 $\frac{1}{2}$		
Othonninae				5	2		1						21 $\frac{1}{2}$	2	
		Total		4	7	24	2		7 $\frac{1}{2}$	5 $\frac{1}{2}$	1				21 $\frac{1}{2}$	2	
Calenduleae						3		5		1 $\frac{1}{2}$						1 $\frac{1}{2}$	
Arctotideae.																	
Arctotidinae				1						7					
Gorteriinae										1			5	2	
Gundeliinae										1			1		
		Total				1						9			6	2	
Cynareae.																	
Echinopsidinae											2				
Carlininae											1		7		
Carduinae											7 $\frac{1}{2}$		10 $\frac{1}{2}$		
Centaureinae											5		6		
		Total											15 $\frac{1}{2}$		23 $\frac{1}{2}$		
Mutisieae.																	
Barnadesiinae										2					
Onoseridinae				2			1 $\frac{1}{2}$			6 $\frac{1}{2}$					
Gochnatiinae															
Gerberinae										13	1	1	7		
Nassauviinae				17						8			2		
		Total				19			1 $\frac{1}{2}$			1					
Cichorieae.																	
Scolyminae		1													
Dendroseridinae		2													
Hyoseridinae		12													
Lapsaninae		3													
Rhagadiolinae		5													
Crepidinae		8													
Hieraciinae		3													
Hypochaeridinae		8													
Lactucinae		14													
Scorzonerinae		13													
		Total		69													

columns. If, however, the genus shows three types of styles it is again counted $\frac{1}{2}$ in each column, and as it is only large genera which show three forms it is not altogether wrong to represent such a genus by a total of $1\frac{1}{2}$ in the table.

Type I is the characteristic style of the ordinary ray florets in all the tribes in which these occur and is noted in the table only in the Cichorieæ where it occurs without exception in every genus. It may be observed that the totals for the tribes do not quite agree with those given previously (61, p. 466), but further study has revealed differences, distinctions and intermediate forms unrecognised in the previous analysis.

The main points to be noted in Table VI are the following:—

Type IV is the only one which occurs in all except the specialised tribes;

Among the tribes in which type IV occurs the Astereæ, Heliantheæ and Heleniæ are mainly types VII and VIII, so that they can scarcely be regarded as primitive in this respect; similarly the Inuleæ have many genera of type XII; the Arctotideæ and Mutisieæ have a considerable proportion of types IX and XII. This leaves the Anthemideæ, an obviously specialised group, and the Senecioneæ, which, while showing a large number of genera with type IV, has quite a number of other types, thus showing the characteristics of a primitive plexus, *i.e.*, a large percentage of primitive forms with an extended variability especially in the centre of the plexus (in this case the Senecioninæ), and a specialisation in the outer groups of the plexus (the Liabinæ and Tussilagininæ);

The occurrence of type III in the Senecioneæ and its exclusive predominance in the Liabinæ and Vernoniæ;

The monomodal curve of complexity, 6, 34, 44, 12, in the Astereæ and the passage of type VIIa of the Homochrominæ into type II of the Eupatorieæ;

The close relationship between types IV and XII and their occurrence in about equal numbers in the Inuleæ;

The similar close relationship between types IV and VII and their occurrence in about equal numbers in the Heleniæ;

The almost exclusive predominance of type IV in the Nassauviinæ.

These and other less obvious points will be discussed later in conjunction with others elucidated by the analysis of the stamens.

C. STAMENS OF THE COMPOSITÆ.

The stamens are five in number, and usually have the anthers syngenesious. As described by Cassini (13) the stamen is composed of the filament, which may be glabrous or hairy, the anther, the connective, the apical appendage, the basal appendages, the pollen and a prolongation of the connective below the anther to form the "article anthérifère." It is certain that in many species there is an abrupt change in the tissue of the stalk of the anther, but it is not always to be observed, and Bentham agrees with later authors in omitting it from his generic diagnoses. Cassini, however, was so impressed with this structure that he considered that the character of the family does not lie in the syngenesious anthers but in the "article anthérifère," and suggests (13, p. 139) that the family would be more appropriately known as the *Androtomæ* than as the *Synanthereæ*. This suggestion of alternative designations was Cassini's chief weakness as a botanist.

The apical appendage is practically always present, but the basal appendages may be absent, or may undergo modifications which have been used by Cassini, Bentham and others to characterise genera, sub-tribes or even tribes, and it is the form and degree of development of the basal appendages that give some clues as to the evolution of the family.

Types of Stamens.

The numerous varieties of stamens can be reduced to sixteen general types as follows:—

Type 1. Apical appendage absent; basal appendages absent; both base and apex of each anther lobe rounded and polliniferous (Fig. 6, 1).

Type 2. Apical appendage present, basal appendages absent; base of anther lobes truncate (Fig. 6, 2).

Type 3. Apical appendage present, basal appendages absent; base of anther lobes rounded and polliniferous (Fig. 6, 3).

Type 4. Apical appendage present; basal appendages very small, non-polliniferous auricles (Fig. 6, 4). In one genus, *Eleutheranthera*, this type occurs with the apical appendage absent as in Fig. 6, 4a.

Type 5. Apical appendage present; basal appendages more distinct as obtuse, non-polliniferous auricles (Fig. 6, 5).

Type 6. As type 5 but with basal appendages acute (Fig. 6, 6).

In a few cases the apical appendage is elongated as in 6a, in others the basal appendages are elongated as in 6b.

Type 7. As type 6, but with the auricles of contiguous auricles connate (Fig. 6, 7).

Type 8. Apical appendage present; basal appendages acute auricles, mucronate or prolonged into small tails (Fig. 6, 8).

Type 9. As type 8, but with the auricles and tails of contiguous anthers connate (Fig. 6, 9).

Type 10. Similar to type 8, but with longer tails (Fig. 6, 10).

Type 11. Apical appendage elongated; basal appendages acute, elongated auricles prolonged into tails (Fig. 6, 11).

Type 12. Similar to type 9, but with considerably longer tails (Fig. 6, 12).

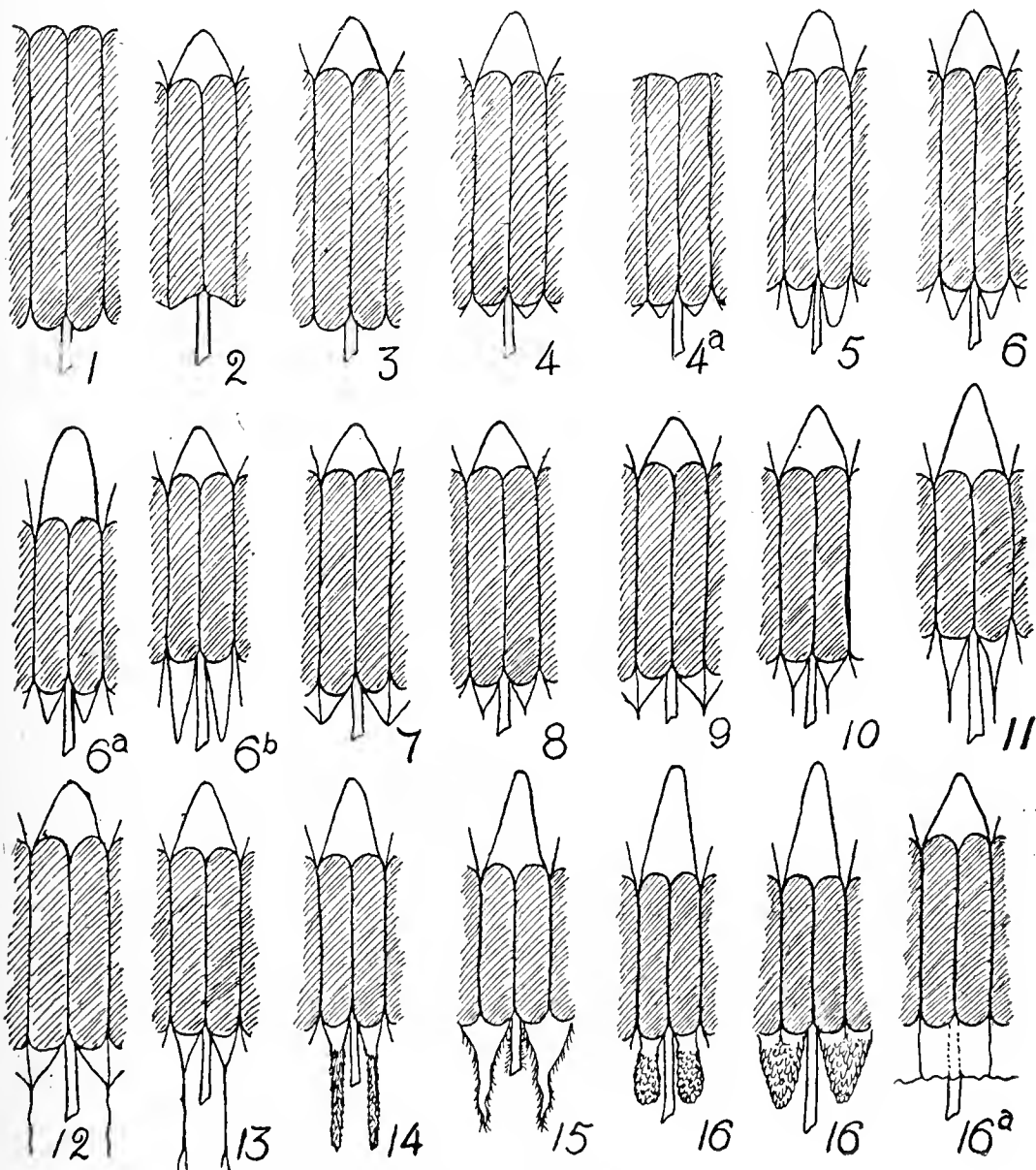


FIG. 6. Stamen forms in the Compositæ.

Type 13. Apical appendage present; basal appendages acute; auricles with long tails divided into two or more filaments (Fig. 6, 13).

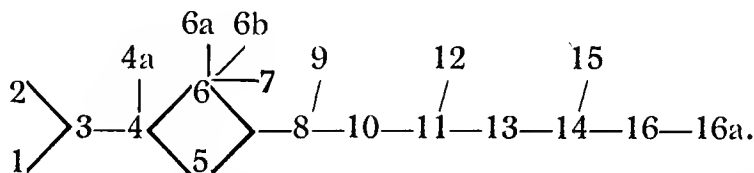
Type 14. Apical appendage elongated (oblong not triangular); basal appendages obtuse; auricles with long, flattened, lacerate tails (Fig. 6, 14).

Type 15. Similar to type 14, but apical appendage more elongated and tails of contiguous anthers connate (Fig. 6, 15).

Type 16. Apical appendage more elongated than in any other type; basal appendages flattened, elaborately lacerate tails, free or those of contiguous anthers connate (Fig. 6, 16). In one genus, *Tricholepis*, the fusion of the anther tails has been carried further, giving a continuous, membranous cylinder around the base of the anther tube (Fig. 6, 16a).

Analysis of the Stamens of Compositæ.

Analysis of the occurrence in the family of these various types of stamens show that type 1 is a special type occurring only in the specialised sub-tribe, *Piqueriinæ*, of the *Eupatoriæ*; type 2 is likewise a special form occurring in a few genera only, while type 3 occurs in a large number of genera belonging to eleven of the thirteen tribes. Type 4 also occurs in most of the tribes but is much less common than type 3, which is obviously the primitive and characteristic stamen for the family. Given type 3 as the basal form it is easy to see that types 1 and 2 are reduced forms while the others form a progressive series which may be given diagrammatically thus:—



Keeping this evolution of form in mind we can now analyse the composition of the tribes and sub-tribes of the family as regards their stamens in the same way as the styles have been analysed.

The main points to be noted in Table VII are the following:—

The primitive character of type 3, as already pointed out;

Type 3 occurs in all the tribes except the *Vernoniæ* and *Cynaræ*; it is predominant in the *Astereæ*, *Eupatoriæ*, *Anthemideæ* and *Senecioneæ*;

Type 4 occurs in considerable proportion, with type 3 predominant in the *Heliantheæ* and *Heleniæ*;

TABLE VII. *Analysis of Stamen-forms in the Compositæ.*

TRIBES & SUB-TRIBES.	TYPES.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Vernonieae.																
Vernoniinae ...					18 $\frac{1}{2}$	3	3			4 $\frac{1}{2}$				1		
Lychnophorinae ...					9 $\frac{1}{2}$	2	3 $\frac{1}{2}$				1					
Total					28	5	3 $\frac{1}{2}$			4 $\frac{1}{2}$	1			1		
Eupatorieae.																
Piqueriinae ...	8															
Ageratinae ...		2	21													
Adenostylinae ...			10													
Total	8	2	31													
Astereae.																
Homochrominae ...		2	29													
Grangeinae ...		2	4													
Bellidinae ...			12 $\frac{1}{2}$		1											
Heterochrominae ...		2	37 $\frac{1}{2}$	1						1						
Conyzinae ...		1 $\frac{1}{2}$	8 $\frac{1}{2}$	1												
Baccharidinae ...		1 $\frac{1}{2}$	2 $\frac{1}{2}$													
Total	8	94	2		1					1						
Inuleae.																
Tarchonanthinae ...				1		1			1 $\frac{1}{2}$	34	1	3				
Plucheinae ...						1				7		9 $\frac{1}{2}$		1		
Filagininae ...			1									3				
Gnaphaliinae ...			1 $\frac{1}{2}$							44 $\frac{1}{2}$		3	5 $\frac{1}{2}$	1 $\frac{1}{2}$		
Angianthinae ...										7			3	2		
Relbaniinae ...										6 $\frac{1}{2}$		3	2 $\frac{1}{2}$	2		
Athrixiinae ...										3 $\frac{1}{2}$		1	2 $\frac{1}{2}$			
Inulinae ...										10		3	5	6		
Buphthalminae ...			1							9		6	3			
Total			3 $\frac{1}{2}$	1		2			1 $\frac{1}{2}$	91	1	31 $\frac{1}{2}$	21 $\frac{1}{2}$	12 $\frac{1}{2}$		
Heliantheae.																
Lagasceinae ...					1											
Milleriinae ...			7	2 $\frac{1}{2}$		1 $\frac{1}{2}$										
Melampodiinae ...	1	13 $\frac{1}{2}$	7 $\frac{1}{2}$													
Ambrosiinae ...			9													
Petrobiinae ...					3											
Zinniinae ...			5 $\frac{1}{2}$	1												
Verbesininae ...	5	29	23 $\frac{1}{2}$	2												
Coreopsidinae ...	1 $\frac{1}{2}$	13	2 $\frac{1}{2}$	1	1											
Galinsoginae ...	1		7													
Madiinae ...	1 $\frac{1}{2}$	5 $\frac{1}{2}$														
Total	9 $\frac{1}{2}$	83 $\frac{1}{2}$	43 $\frac{1}{2}$	7 $\frac{1}{2}$	1 $\frac{1}{2}$											
Helenieae.																
Jaumeinae ...			5		2											
Baeriinae ...	1 $\frac{1}{2}$	19	13 $\frac{1}{2}$	1												
Flaveriinae ...			3													
Tagetinae ...	1	11 $\frac{1}{2}$	1 $\frac{1}{2}$													
Heleniinae ...			4 $\frac{1}{2}$		2											
Total	2 $\frac{1}{2}$	39	19 $\frac{1}{2}$	3	2											
Anthemideae.																
Anthemidinae ...	1	12 $\frac{1}{2}$	1 $\frac{1}{2}$													
Chrysanthemidinae ...	1 $\frac{1}{2}$	33	2 $\frac{1}{2}$													
Total	2 $\frac{1}{2}$	45 $\frac{1}{2}$	4													
Senecioneae.																
Liabinae ...		1	1	1		1		1	1							
Tussilaginatae ...		2	2			1 $\frac{1}{2}$	1 $\frac{1}{2}$			1						
Senecioninae ...	1 $\frac{1}{2}$	21	8			2		1 $\frac{1}{2}$								
Othonninae ...		6 $\frac{1}{2}$	1 $\frac{1}{2}$													
Total	2	30 $\frac{1}{2}$	12 $\frac{1}{2}$	1	1 $\frac{1}{2}$	2	3 $\frac{1}{2}$	1	1	3						
Calenduleae.			3									1				
Arctotideae.																
Arctotidinae ...		2		3 $\frac{1}{2}$	2	1				2						
Gorteriinae ...		1	2	1 $\frac{1}{2}$	1 $\frac{1}{2}$			1								
Gundelinae ...				2												
Total		3	2	7	3 $\frac{1}{2}$	1	1			2						
Cynareae.																
Echinopsidinae ...									2			1			1	1
Carlininae ...									1 $\frac{1}{2}$	1 $\frac{1}{2}$		4		1	4 $\frac{1}{2}$	6
Carduinae ...									2			3 $\frac{1}{2}$			4 $\frac{1}{2}$	1 $\frac{1}{2}$
Centaureinae ...									5 $\frac{1}{2}$	1 $\frac{1}{2}$		8		1	10 $\frac{1}{2}$	13 $\frac{1}{2}$
Total																
Mutisieae.																
Barnadesiinae ...		1 $\frac{1}{2}$	1													
Onoseridinae ...						1					3 $\frac{1}{2}$	1	6	11	1 $\frac{1}{2}$	1
Gochnatiainae ...											6	1 $\frac{1}{2}$				
Gerberinae ...										1	3	1	5 $\frac{1}{2}$			
Nassauviinae ...										1	11	1 $\frac{1}{2}$	4	26 $\frac{1}{2}$	1 $\frac{1}{2}$	1
Total		1 $\frac{1}{2}$	1			1				2	23 $\frac{1}{2}$	2	2 $\frac{1}{2}$			
Cichorieae.																
Scolyminae ...								1								
Dendroseridinae ...						1 $\frac{1}{2}$		1								
Hyoseridinae ...			1		1	5		6								
Lapsaninae ...						1 $\frac{1}{2}$		1 $\frac{1}{2}$								
Rhagadiolinae ...						2 $\frac{1}{2}$		2 $\frac{1}{2}$								
Crepidinae ...						4		4								
Hieraciinae ...						1		2								
Hypochaeridinae ...						2 $\frac{1}{2}$		4 $\frac{1}{2}$								
Lactucinae ...						6		8								
Scorzonerinae ...						6		7								
Total			1		1	30		37								

A predominance of type 5 in the Vernoniæ is combined with a proportion of higher forms ;

A predominance of type 3 in the Eupatoriæ is combined with the occurrence of a few genera of Type 2, and an exclusive predominance of type 1 in the Piquerinæ :

A predominance of type 3 in the Astereæ is associated with a proportion of type 2 and a very few higher forms ;

The predominance of higher forms, types 10-14, in the Inuleæ and types 9-16 in the Cynareæ ;

The circumscribed variation in the types represented in the Heliantheæ (2-6), in the Heleniæ (2-6), in the Anthemideæ (2-4) and in the Cichorieæ (3, 5, 6, 8) ;

The considerable range of variation (3-16) in the Mutisiæ ;

The Senecioneæ again show the characteristics of a primitive plexus, but with the specialisation of the outer groups not shown as markedly as in the styles ;

The Calenduleæ and the Arctotideæ show a range of variation very similar to that of the Senecioneæ but without the predominance of type 3.

D. FUNCTION OF APPENDAGES OF STYLES AND STAMENS.

The biological significance of the hairs and appendages of the styles was appreciated by Cassini (13) and all subsequent authors, but the biological significance of the appendages of the stamens was elucidated for the first time in 1915 by the writer (61). Both Cassini and Bentham as shown above (Section A, Chap. II) recognised the value of these structures for the purposes of classification, but Cassini makes no mention of a possible function, while Bentham considers them to be "of little or no functional or homological importance."

That the predominant tendency in the Compositæ is economy has been emphasised (61-62), and "since the highest development of these appendages is found in the same groups with the greatest complexity in the structure of the style, it becomes highly probable that the appendages have quite a definite function" (61, p. 459). Economy in the polliniferous tissue was then considered and a new significance attached to the sweeping hairs of the styles and their efficiency in removing all the pollen grains from the anther tube. The main point can best be made clear by a quotation from the original article (61, p. 460). "The functions of the staminal appendages, hitherto obscure, become more obvious, for with the

corolla tube of a given length and the stamens in proportion, the amount of pollen produced can be reduced and the staminal tube remain the same length by the production of a membranaceous prolongation at the apex of each anther. This is a very simple method of reducing the polliniferous tissue while preserving efficiency of the staminal tube in the pollen-presentation mechanism. The function of the basal appendages is also made clear, since a tube terminating in ten more or less hemispherical lobes, as in type 3, could not be closed entirely by the style unless that organ actually entered the tube for some distance, in which case the pollen in that part of the sac past which the style had grown would be more or less lost for pollination purposes, unless it was swept up the tube by hairs situated lower down on the style. If the apex of the style merely reached to the lobes when the anthers dehisced, some of the pollen would fall through the interstices to the bottom of the corolla tube and thus be lost. If, however, the hemispherical lobes were prolonged into flattened auricles or ciliate tails or more elaborate appendages, the style, with or without appendages, could close the lower end of the staminal tube completely without encroaching on the polliniferous region, and thus no pollen would be wasted as far as the pollen-presentation mechanism was concerned."

From the further extended study of many forms there is no doubt that the above is the correct explanation of the variations in structure of the styles and stamens, and it is considered proved that these appendages and variations are the several modes of expression of a tendency in the Compositæ to economy of pollen, which is limited only by the biological necessity of providing sufficient pollen to ensure fertilisation.

Except in the relatively primitive groups, the Anthemideæ and Senecioneæ, relatively inefficient anther tubes are combined with efficient styles, as in the Eupatorieæ, Astereæ, Heliantheæ and Heleniæ, or relatively inefficient styles are combined with efficient anther tubes, as in the Vernoniæ, Inuleæ, Calenduleæ, Arctotideæ and Cichorieæ, or both styles and anther tubes are efficient, as in the Mutisieæ and Cynareæ.

Another point which is brought out by further study of the structure of the stamen is that Cassini's interpretation of the apical appendage and the "article anthérifère" as continuations of the connective is supported by the fact that the structure of these three parts of the stamen is very similar. This leads to the con-

clusion that both the apical appendage and the "article anthérifère," where it occurs, are produced by a contraction of the polliniferous region of the anther, *i.e.*, by a real sterilisation of potentially sporogenous tissue.

E. THE PHYLOGENETIC SIGNIFICANCE OF THE STYLES AND STAMENS.

As the complexity of the styles and that of the stamens have been shown to be more or less complementary, it is necessary in the elucidation of the phylogeny of the groups to consider, not so much the two sets of organs separately, as the complexity of the pollen-presentation mechanism as a whole. Each tribe will, therefore, be examined in order to determine the primitive and advanced sub-tribes and these will also be examined in order to determine their relations with one another throughout the family as far as it is possible to elucidate this from the pollen-presentation mechanism alone.

As the *Senecioneæ* appear from the analysis of both styles and stamens to be the primitive tribe it will be considered first. The *Liabinaæ* are undoubtedly specialised in their style (Type III) but the types of stamens vary widely (2-9). The chief genus, *Liabum*, has comparatively simple stamens, type 2 or 6, and in the sub-tribe as a whole we get somewhat the same range of variation in the stamens as occurs in the *Vernoniææ*.

Three of the chief genera, *Homogyne*, *Cremanthodium*, *Alciope* and also *Luina*, in the *Tussilagininæ*, show styles of type II. In *Luina* this is combined with stamens of types 8 or 10 and in *Alciope* with types 6 or 7, giving in both cases a relatively complex pollen-presentation mechanism. In *Homogyne* and *Cremanthodium* style type II occurs with stamens type 3 or 4, so that here the mechanism is less complex. Now *Cremanthodium*, although separated from *Senecio* as a distinct genus by Bentham and other systematists, is included in that genus by Franchet (23) who places it in the group *Ligularia* of *Senecio*, dividing that group into A-*Cremanthodium* and B-*En-ligularia*. It is really a mere matter of opinion, as even those who uphold the genus admit that it passes into the *Ligularia* group through such species as *Cremanthodium plantagineum*, Moor., which sometimes shows as many as five capitula instead of the solitary one which characterises the genus, and also through *Senecio calthæfolius*, Hk.f. (= *Cremanthodium Hookeri*, C.B.C.), which shows from one to four capitula. The only other distinction lies in the "nodding"

of the capitulum! The Tussilagininæ are, therefore, very intimately connected with the genus *Senecio*, which itself shows type VII styles not infrequently, and more rarely, as in some sub-sections of the *Ligularia* group, type VIII, thus leading up to type II in *Cremanthodium*.

The Othonninæ cannot be distinguished from the Senecioninæ by either styles or stamens, both being comparatively simple.

The Senecioninæ in both stamens and styles shows the characteristics of a primitive group. Type IV styles are combined in most genera with type 3 stamens, a simple and primitive pollen-presentation mechanism. *Senecio* itself, however, sometimes shows styles of types VII and VIII and sometimes stamens of types 4 or 8 but these two elaborations rarely, if ever, occur in the same species. They are, indeed, associated with two very different habits. Other closely allied genera always show type VII or type VIII in their styles, others always show type XII styles, which type also occurs in some species of *Senecio*.

It is not possible at this point to distinguish which is the more primitive of the two sub-tribes of the Vernoniæ but most of the large genera with simple stamens occur in the Vernoniinæ. *Vernonia* itself shows both type 5 and type 10.

In the Eupatoriæ the style is of a uniform type as in the Vernoniæ, so that the relative complexity of the pollen-presentation mechanism depends on the type of stamen. The simplest type of stamen occurs in the Piqueriinæ and nowhere else in the family. That this is a reduced, not a primitive type, is rendered probable by its occurrence only in this small and somewhat specialised group and is proved by the series of forms previously figured (61, Figs. 3-6). The normal apical appendage as in *Eupatorium cannabinum* (61, Fig. 6) becomes truncate and obviously reduced in *Sclerolepis* (61, Fig. 5) which is the genus in the Ageratinæ next the Piqueriinæ. In *Adenostemma viscosum* (61, Fig. 4) and other species of the chief genus in the Piqueriinæ the line of dehiscence does not extend to the apex of the anther when dehiscence is completed and the apical indehiscent region carries out the function of the ordinary apical appendage. In the other genera (61, Fig. 3) dehiscence is carried to the apex and the abortion of the apical appendage is complete. It is scarcely possible to distinguish at the present stage between the other two sub-tribes, but the complete uniformity in the stamens as in the styles of the Adenostylinæ indicates a probably specialised group.

The simplest pollen-presentation mechanism in the *Astereæ* is that of the *Bellidinæ* and *Heterochrominæ*, where stamens of type 3 are combined with styles of type VII in a number of genera. Styles of type VIII occur largely in the other sub-tribes and type VIIa occurs in a notable percentage of the *Homochrominæ*, the sub-tribe placed next the *Eupatoriæ* by Bentham.

Considering next the *Inuleæ*, the *Gnaphaliinæ* is the only sub-tribe which shows a preponderance of simple (type IV) styles with a preponderance of comparatively simple stamens (type 10). The sub-tribe is divided into two series, the second of which, the *Helichryseæ*, has by much the larger proportion of simple type pollen-presentation mechanisms; the first series, the *Eugnaphalieæ* has a larger proportion of styles of type XII and of stamens of types 12 and 13. The other sub-tribes may be supposed to have come off as in Fig. 7, taking into consideration the predominance of type XII styles in the *Inulinæ* and *Bupthalthinæ*, their development in the *Athrixinæ*; the occurrence of simple stamens in the *Plucheinæ* and *Filaginæ* with a considerable percentage of type XII styles; the occurrence of type V styles in the *Tarchonanthinæ*, and a larger proportion of type 10 stamens in the *Angianthinæ* than in the *Relhantiinæ*.

The largest proportion of simple pollen-presentation mechanisms in the *Heliantheæ* occurs in the *Verbesininæ*. Of the other sub-tribes the *Lagasceinæ*, *Milleriinæ*, *Melampodiinæ*, *Ambrosiinæ* and *Petrobiinæ* are obviously somewhat restricted or specialised, while the *Zinniinæ*, *Coreopsidinæ* and *Madiinæ* are closely allied to the *Verbesininæ*, but are on the whole higher in their style forms than the above-mentioned groups. The *Galinsoginæ* show a variation in the style forms more allied to that of the *Heleniæ* and are closely allied to but slightly higher than the *Verbesininæ* in their stamen forms.

In the *Heleniæ* a large proportion of higher type stamens is shown by the *Jaumeinæ* (type 5), the *Baeriinæ* (types 4-5) and the *Heleniinæ* (types 4 and 6). This is combined in the *Jaumeinæ* and *Baeriinæ* with a preponderance of higher type styles (types VII and VIII). The *Flaveriinæ* are obviously a specially simple group, while the *Tagetinæ* show a preponderance of simplicity with a range of variation which covers that of most of the other sub-tribes.

The *Anthemideæ* are a comparatively simple but special group, closely connected with the *Senecioninæ* and with practically no difference in the development of the pollen-presentation mechanism

in the two sub-tribes. The Calenduleæ are similarly closely connected with the Senecioninæ by their range of variation in both styles and stamens; the characteristic style of the tribe (type V) is easily derived from type IV.

The Arctotideæ show a considerable proportion of styles of type XII; the range of variation is very similar to that of the Senecioninæ and the stamens are considerably simpler than those of the majority of the Inuleæ, so that a derivation of the Arctotideæ from those genera (*Senecio* and *Erechthites*) in the Senecioneæ which show type XII styles seems more probable than one from the Inuleæ as suggested by the styles. Also *Ursinia* is the only genus in the tribe which shows a type IV style, and as it is one of the largest genera and shows type 3 stamens in addition, it seems to be another possible primitive genus in the group. Indeed, the tribe is markedly divided into two groups on the style. The occurrence of stamens of types 8 and 10 in the Gorteriinæ with type XII styles seems to indicate that this sub-tribe has arisen from the Inuleæ (from either the Plucheinæ or more probably the Gnaphaliinæ) and has then given rise to the more specialised Gundeliinæ. The Arctotidinæ seem to be more probably derived from the Senecioninæ through *Ursinia* with a IV-3 pollen-presentation mechanism. This is a point which requires other characters for its decision.

In the Cynareæ the Echinopsidinæ are obviously specialised, while the large percentage of advanced styles (type XI) and advanced stamens (type 16) in the Carlininæ and Carduinæ indicates that these are more advanced groups than the Centaureinæ, where there is a smaller percentage of type XI styles and where the stamens are generally type 12 or type 15.

The almost exclusive predominance of type IV styles in the Nassauviinæ clearly indicates an affinity for that sub-tribe with the Senecioneæ; the Onoseridinæ shows an affinity with the Nassauviinæ in the styles, while type 15 or 16 stamens are absent from both sub-tribes; the predominance of type 11 stamens in the Nassauviinæ makes the usual pollen-presentation mechanism of that group the simplest in the Mutisieæ. The Barnadesiinæ is a simple, special group, the usual combination being IX-3. The occurrence of styles of types X and XI and stamens of types 15 and 16 in the Gochnatiinæ places this group higher than the Gerberinæ, but as type XII styles are also present and as the stamens are of the higher types a possible affinity of these two sub-tribes with the Inuleæ is indicated,

It is very probable that most of the sub-tribes of the Cichoriæ are artificial, therefore it is not considered desirable to discuss their affinities. The derivation of type I styles from type IV has already been mentioned and as the stamens are of a relatively simple type a derivation of the Cichoriæ from the Senecioninæ is rendered probable.

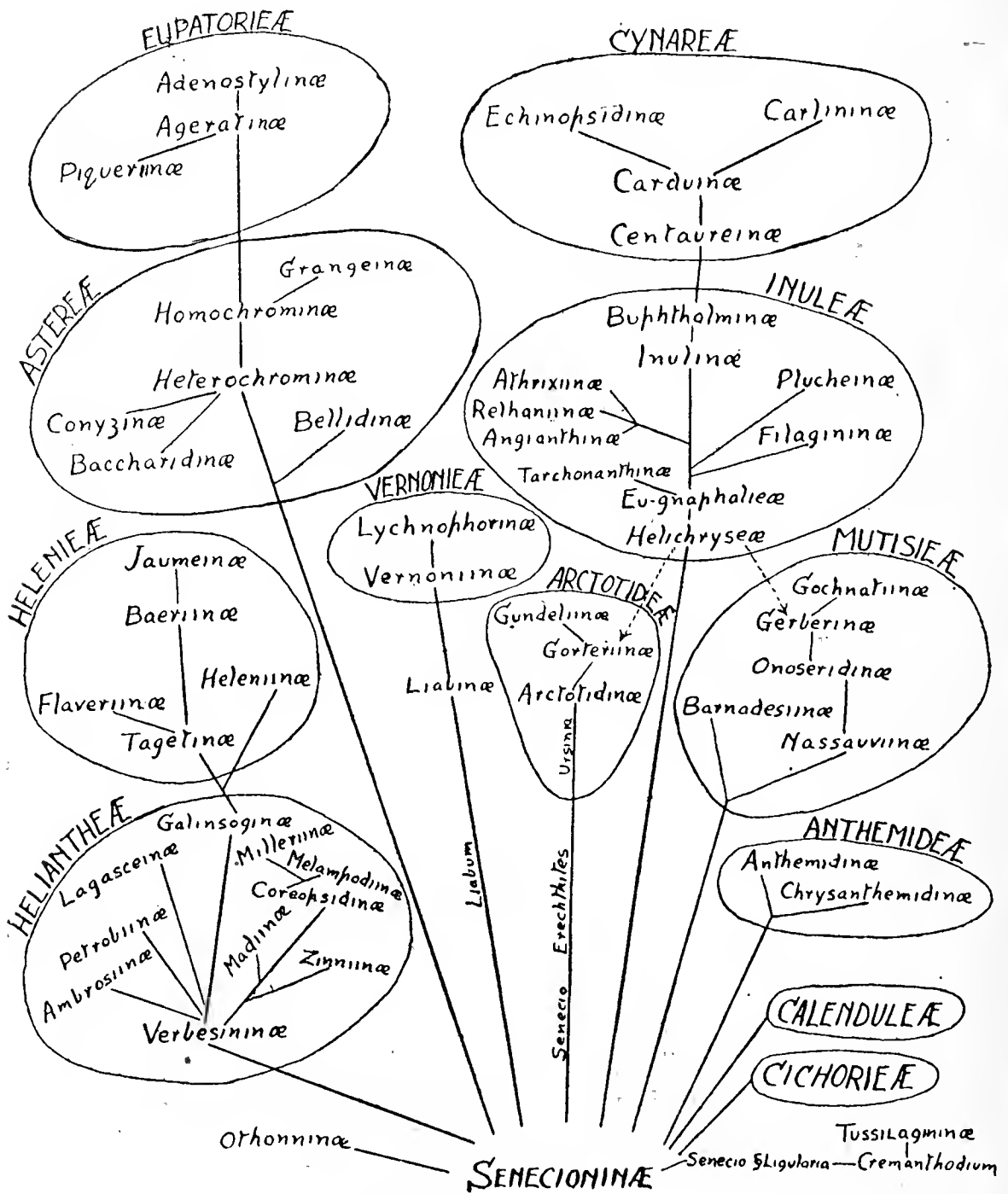


FIG. 7. Phyletic lines in the Compositæ as indicated by the Pollen presentation Mechanism.

All the affinities indicated by the preceding examination of the pollen-presentation mechanism of the various tribes and sub-tribes are shown in Fig. 7, including the somewhat problematical relationships between the Inuleæ and the Gorteriinæ and Gerberinæ, which may be no more than examples of parallel evolution.

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THE ENDOTROPHIC MYCORHIZA OF ERICACEÆ.

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[WITH FOUR FIGURES IN THE TEXT].

THAT there is a necessary symbiosis between Ericaceæ and endophytes, has been recently demonstrated by Dr. Chevely Rayner as far as *Calluna vulgaris* is concerned (1). Ovarial infection was proved by Rayner for *Calluna*, and reported by her for *Vaccinium*.¹ This makes it specially interesting to make a close study (3) of that very peculiar Ericaceous plant *Arbutus Unedo*, a shrub very common in Mediterranean countries and also in the S.W. of France,² but of which the mycorhiza does not seem to have been investigated so far, although in view of the edaphic peculiarities of *Arbutus*, it is certainly of considerable ecological interest.

The roots of *Arbutus* are clothed with a dense mantle of hyphæ. These are protected with a thick greyish membrane (Fig. I). Some of the branches they form have but a thin yellow membrane, and penetrate further into the tissues of the roots, whence they invade the whole plant. Haustoria are formed in the living cells of the host. These latter, as a rule, do not react, but the cytoplasm first becomes granular and then dwindles as the fungus develops. The cortex of the root often consists of empty cells, for here both the host cells and the invading fungus may ultimately die.

FUNGUS-TUBERCLES.

Rootlets which have been inoculated early cease to grow apically,³ and develop into small pear-shaped tubercles. Nearly all the epidermal cells develop into root-hairs. Algæ and bacteria collect around them and form a mucus which stains readily with eosin. The fungus grows not only on their surface, but penetrates and invades the external layer of the cortex (Fig. I, 3). The base of the shoot develops into a very large tubercle from which many lateral

¹ Ovarial infection of *Vaccinium* was not admitted by Stahl, and no mention of it is made in the most recent American work on the subject (2).

² In the latter country this shrub is rather strangely limited to the old Pine forests of Arcachon and La Teste (60 kilometres south of Bordeaux) where the sand is covered by a peaty layer more than 20 cms. deep. *Arbutus* is never met with in the neighbouring Pine forests that have been grown in the sandy dunes of Gascogne for the last century. This strict limitation has puzzled many naturalists and has been the object of several notes (4).

³ This, which was also observed in the cases of *Vaccinium* and of *Arbutus*, supports P. Vuillemin in the belief that even the tubercles of leguminous plants are abortive roots.

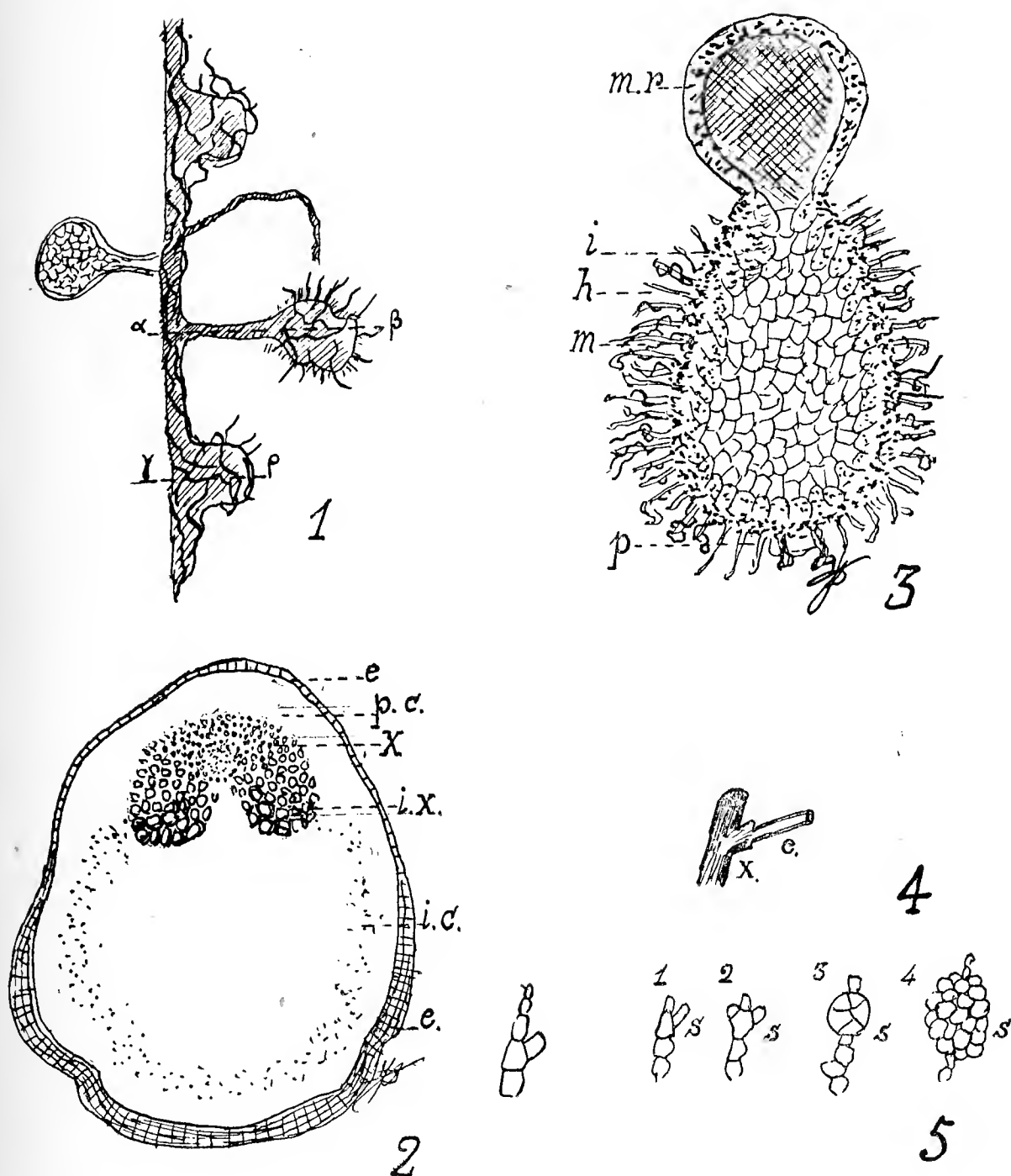


FIG. 1. Fungus tubercles on roots of *Arbutus Unedo*. (1) Roots with fungus-tubercles. (2) Section of lateral root-tubercle along plane $\gamma\rho$ in 1. *e.*, epidermis; *p.c.*, parenchymatous cortex; *x.*, xylem; *i.x.*, infected xylem; *i.c.*, infected cortex; bounded externally by layer of cork. (3) Section of infected rootlet along plane $\alpha\beta$ in 1. *m.r.*, mother root; *m.* mucus formed by bacteria; *i.* infected cells; *h.*, hyphæ of mycorrhiza; *p.*, root hairs. (4) Root fungus. *x.*, brown hyphæ, exogenous; *e.*, light yellow hyphæ penetrating root. (5) Fungus forming spores.

stems spring. Sections show great development of xylem (chiefly of medullary rays). This tubercle stores large quantities of reserve material, such as tannin, which exists in the cortex; tiny fatty drops are seen in all cells while the medullary tract and rays are crowded

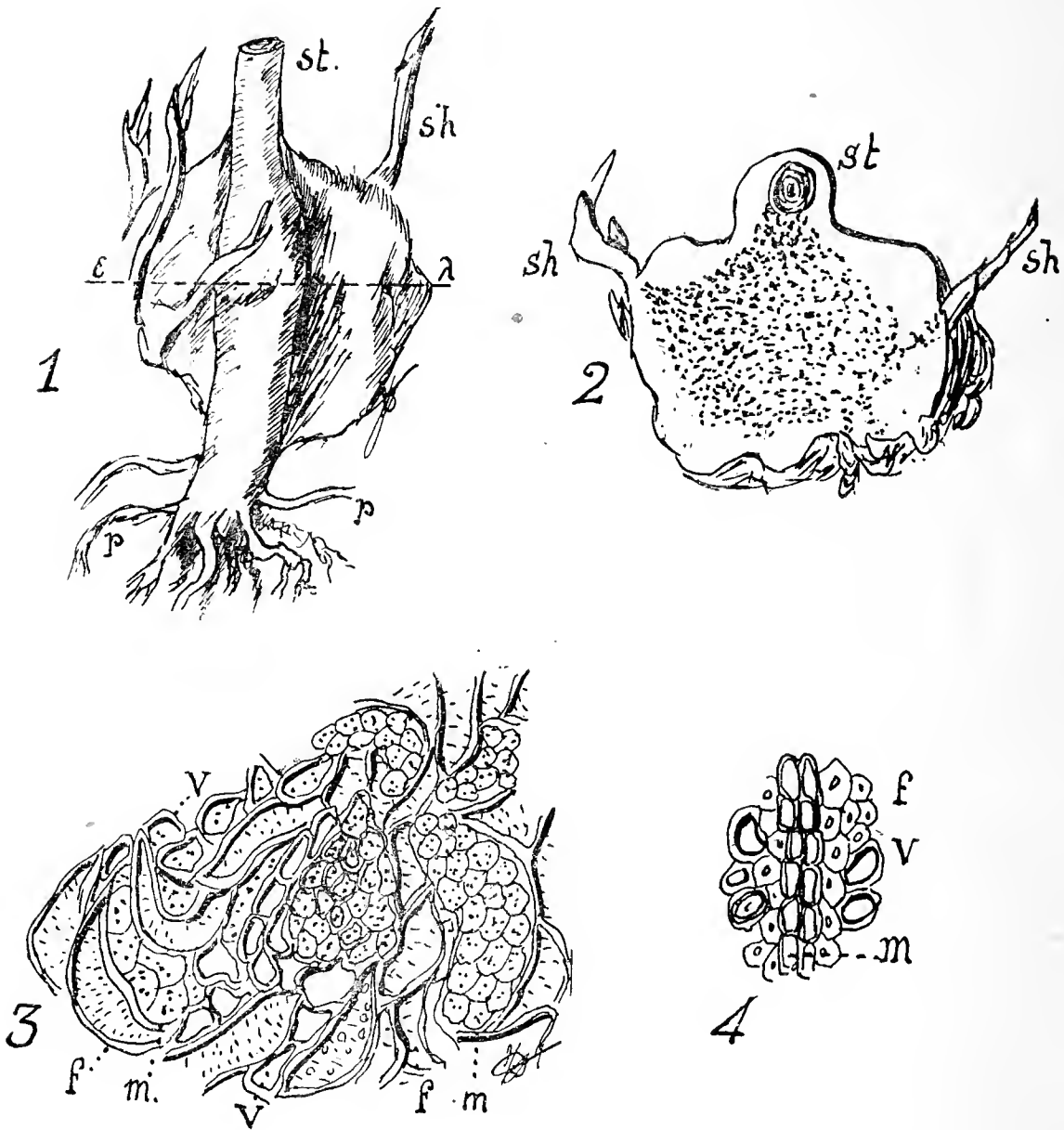


FIG. II. Stem tubercle. (1) External view. ¹St., stem; sh., shoots; r., roots. (2) Section along plane ελ in 1, showing area of starch grains. (3) A portion of 2 × 500. f., wood fibres, v., vessels, m., medullary ray cells crowded with starch grains. (4) Normal medullary ray from stem.

with big starch grains (Fig. II, 1, 2, 3, 4). Here and there tubercles appear laterally on the roots or on the shoots, where they may cause the epidermis to burst, and so result in a wound (Fig. III, 1). In all these tubercles the fungus is rather conspicuous, but it can be traced by careful investigation¹ in all the tissues of the roots, shoots, leaves and fruits.

The inflorescences of *Arbutus* contain numerous flowers; but

¹ Sections of fresh tissues should be examined in *Lactophenol* or in a water solution of iodine and iodide of potassium. Sections may also be stained in eosin solution and then washed out in a solution of hydrochloric acid until the fungus appears clearly differentiated.

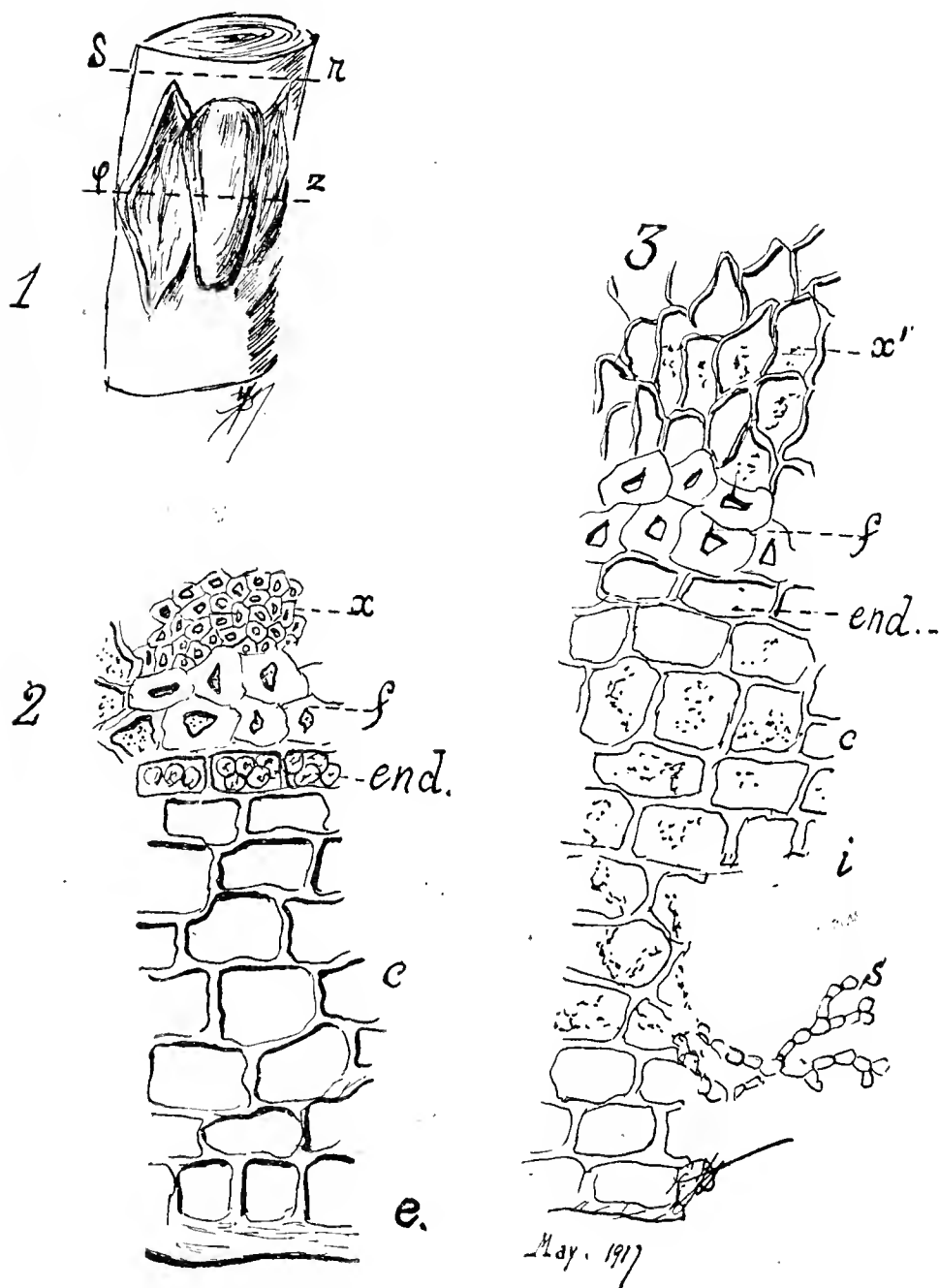


FIG. III. (1) Stem tubercle. (2) Transverse sections of normal stem, along plane $\delta\pi$ in 1. (3) Transverse section of stem tubercle, along plane ϕz in 1. x ., phloem; f ., fibres; $end.$, endodermis (crowded with starch in 2, no starch in 3); c ., cortex; e ., epidermis; s ., fungus forming spores in cortex of 3 x' , hypertrophied phloem infested by fungus.

only one (or in any case only a few) comes to seed. It may be supposed that the necessary adjustment between the endophyte and the cells of the ovary is only reached in a comparatively few cases.

When any of the organs of *Arbutus* dry up, the fungus, in these parts, cannot grow any longer as an endophyte, and has to resume the life of a saprophyte. This is a normal occurrence on the corolla and stamens of the *Arbutus* flower, as these parts

remain attached long after fertilisation, but dry up. It may be observed also on all drying leaves. In such situations, instead of the fine light threads which grow in the living tissues of *Arbutus*, the fungus forms its mycelium on the surface of the dried leaves. The hyphæ of such a surface mycelium are large, of a brownish colour, and closely resemble those that grow in the earth outside the roots. There, however, they form numerous conidia or exogenous spores (Fig. I, 5). Such a mycelium is always present on the dried sepals which protect the young fruits; and it also exists on the surface of pollen-grains. Here, then, are two other ways in which the fruit may be inoculated. Ovules may be inoculated from the pollen-grains, or later on, when the fruit matures, it may be inoculated through its surface by the spores or the parts of mycelium which break off from the sepals.

RELATION OF ENDOPHYTE TO HOST.

Roots of *Arbutus* live in a medium teeming with micro-organisms (*Nectria*). Some fungus, living (as a saprophyte) in the ground, penetrates the roots and extends into practically every part of the tree, even into the assimilating and reproductive tissues.

As a rule, the cells of *Arbutus* do not react, and the presence of the fungus is difficult to trace. Infected rootlets, however, very often give rise to special symbiotic organs (*root-tubercles*): here the plant cannot prevent invasion by the fungus, but checks its excessive propagation. This power of resistance may be defeated, and the fungus may become locally parasitic: it then causes the host cell to react and results in *pathological tubercles*. When the host tissues die, either in a normal way by external causes, or as a result of the parasitic attacks of the fungus, the fungus, being no longer provided with a favourable living medium, becomes a *saprophyte*, and forms spores.

Whether the original relation is to be considered as *symbiosis* or as *parasitism* depends upon the pathogenic reaction of the host. In most cases the cells of *Arbutus* do not react, and symbiosis may be considered to exist. Local reactions, however, prove that the fungus may also become a parasite.

General, as well as specific infection is, however, responsible for some of the observed pathogenic reactions: fungal hyphæ and bacteria aggregate around the root tubercles, rusts invade the shoot tubercles, and moulds grow on the dying leaves or sepals. The precise biological rôle of each is not yet known.

METABOLIC EXCHANGES BETWEEN HOST AND ENDOPHYTE.

Whether there is symbiosis or no, depends upon the exchanges of nutritive material between the two associated organisms. "When one plant is parasitic on another," writes MacDougal (5), "such conditions must be present as to cause water to flow from the host to the tissues of the parasite, carrying substances in solution."

In the case of *Arbutus* three parasitic relations may be considered:—

(1) *The fungus draws carbohydrates from the host.*

This is easily proved: a section of the stem is stained by Gram's method, then treated, first with dilute potassium hydrate, secondly with weak hydrochloric acid (Mme. Gatin-Grusewska's method). Starch grains are mostly dissolved, only the central part remaining. It is then possible to see the haustorial organs which the endophyte has sent toward the starch-grains to digest them (Fig. IV).

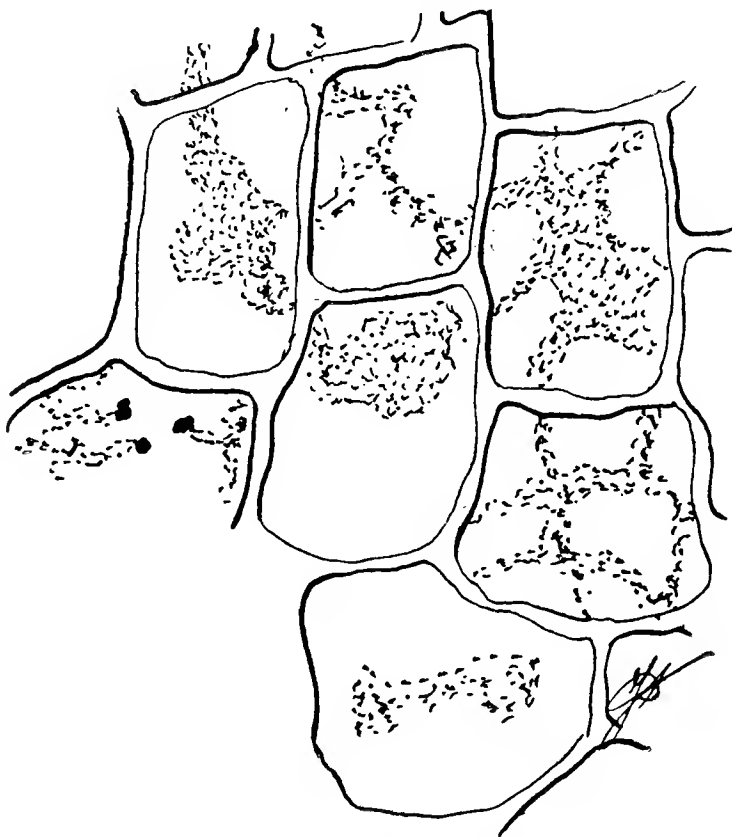


FIG. IV. Endophyte in cells of leaf *Arbutus Unedo*.

(2) The fungus root tubercles of *Arbutus* closely resemble those of leguminous plants, and the inoculated cells are near the surface of the host tissues. So it might be supposed that, here also, the fungus obtains free nitrogen from the atmosphere; but this, of course, can only be proved by cultivation of inoculated *Arbutus* in a soil free from nitrogen.

(3) It is assumed since Frank's researches that the host derives part of its food from the carbon compounds which the mycorrhiza draws from the humus in the soil.

CONCLUSIONS.

The example of *Arbutus* supports Rayner in the belief that symbiosis with endophytes is a general rule, at least, for Orchidaceæ and Ericaceæ.

It shows again that *the so-called symbiosis is a form of parasitism in which an equilibrium exists between the invading power of the fungus and the resisting power of the host*; also, that it is a *reversible* phenomenon—profitable to both symbionts so long as the equilibrium remains balanced; but each is ever ready to assume the character of parasite and bring about the death of the other.

STATION BIOLOGIQUE, ARCACHON, FRANCE.

May, 1917.

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NOTE ON *TARGIONIA HYPOPHYLLA*..

IN 1914 the writer published some of his observations on a form of *Targionia hypophylla* which he had collected at Mussooree (*NEW PHYTOLOGIST*, Vol. XIII, Nos. 6 and 7). Since then the writer has found that the plant is pretty common throughout the whole of Garhwal. The form from Mussooree was described as a distinct variety as it presented several differences from the

type, the chief among which as stated were: (1) the occurrence of antheridia on ordinary shoots and the usual disc-like male shoots; and (2) the absence of tooth-like interlocking processes from the involucreal valves.

In a paper by O'Keeffe in 1915 (NEW PHYTOLOGIST, Vol XIV, Nos. 4 and 5) it has been shown that the peculiar male shoots described in the Himalayan form by the writer also occur in British specimens, so that the character can no longer be said to be peculiar to the former. In the light of the details and figures given in the above mentioned paper it was thought desirable to examine some more Himalayan material of this species as regards the second peculiar character, *i.e.*, the entire margin of the involucreal valves, especially because at the time when the writer published his observations he was under the impression that the teeth on the involucreal valves in the typical specimens were large enough to be visible to the naked eye. A thorough re-examination of material from Mussooree has brought out the following points:—

1. As seen by the naked eye or with a lens the margin of the involucreal valves as a whole is usually quite entire, though sometimes a few small irregular obtuse teeth are present.

2. As seen in microtome sections often the greater part of the valve-margin shows no interlocking processes as figured by O'Keeffe, or the marginal cells are only more or less irregular or disorganised, but a small part of the valves near the dorsal surface shows well developed interlocking processes.

3. In other specimens distinct teeth were present on the whole of the margin.

It appears, therefore, that since the two important characters on which the variety *integerrima* was based are not constant features of the Himalayan form, there is no need of the form being put in a separate variety.

It may also be mentioned that according to the view put forward by the writer as regards the derivation of *Targionia*-like forms from *Marchantia*-like forms by reduction (NEW PHYTOLOGIST, Vol. XIV, No. 1) the interlocking teeth on the involucreal valves of *Targionia* would be the last remnants of the long processes of the fimbriated valves of the involucre of *Marchantia*, and even these teeth are in process of being eliminated.

SHIV RAM KASHYAP.

REVIEW.

"FOSSIL PLANTS." A TEXT-BOOK FOR STUDENTS OF BOTANY AND GEOLOGY. BY A. C. SEWARD, F.R.S. VOL. III.¹ Pteridospermeæ, Cycadofilices, Cordaitales, Cycadophyta. With 253 Illustrations. Cambridge, at the University Press. 1917. Pp. xviii and 656. Price 18/-.

PROFESSOR SEWARD'S great work is now nearing its conclusion, for Vol. IV, completing the book, is already in the Press, and will it is hoped be published before the end of the year. The present volume is dedicated to the memory of the author's friend, Charles René Zeiller, whose death two years ago was so irreparable a loss to Fossil Botany. His portrait forms the frontispiece.

The subject of geographical distribution, on which the author is so well qualified to speak, is reserved for connected treatment in a separate book. As will be seen from the title, the groups of plants described in Vol. III are of the utmost importance, and are perhaps those of the greatest interest in the present position of Palæobotany. While the Pteridosperms, Cycadofilices and Cordaitales were essentially Palæozoic plants, the Cycadophyta were dominant in the Mesozoic period and still survive. It is needless to say that no such ample and connected account of these great classes of plants has appeared before. The volume is thus a contribution of the highest value to the study of the plants of past ages.

The opening chapter (XXVIII) is appropriately devoted to an excellent account of the recent Cycads. The question of the mesarch foliar bundles is discussed, with reference to the primary or secondary nature of the centrifugal portion of the xylem. The point, though interesting, is perhaps less essential than is sometimes supposed, for primary centrifugal xylem tends to become merged in the secondary, as is well shown in the leaf-trace strands of the Cordaitales.

The next chapter is on the Lyginopterideæ. The generic name *Lyginopteris*, Potonié, is adopted instead of the familiar *Lyginodendron*, essentially on the ground that the name *Lyginodendron* "was first used for a specimen which has nothing to do with the plant usually spoken of as *Lyginodendron oldhamium*" (p. 38). This change of nomenclature may be somewhat unwelcome to British palæobotanists, but there are strong grounds for it, especially in view of the fact that Goullie's name *Lyginodendron* is still employed in its original sense, for certain casts of *Lepidodendroid* stems. This double usage is confusing and illegitimate, and it is probably best to make up our minds to adopt the unambiguous name *Lyginopteris*.

Binney's type specimen (1866) was first figured by Dr. Arber in 1902, and his figure is reproduced on p. 39. With reference to the microsporangia, the bilocular sporangium shown in Fig. 407, A,

¹ Vol. II of this work was reviewed in the NEW PHYTOLOGIST in 1910, 9, p. 264. Vol. I was published in 1898.

affords an interesting confirmation of Dr. Kidston's conclusions. The question of the specific identification of Dr. Kidston's specimens with *Sphenopteris Höninghausi* is discussed. Chodat has attributed sporangia of quite a different type to *Lyginopteris*; the reviewer may point out that the pinnules on which such annulate sporangia as those figured by Chodat are borne (*Pteridotheca* spp.) are certainly not identical with the leaflets of *Lyginopteris*.

The evidence for the attribution of the seed *Lagenostoma Lomaxii* to *Lyginopteris* is judiciously summed up, and the conclusion accepted. The possibility of insect agency in the transport of pollen is recognised.

The account of the genus *Heterangium* is well up to date. Dr. Margaret Benson's observations are regarded as almost amounting to a demonstration of continuity between vegetative organs and seeds (*Sphaerostoma*).¹

The family Medulloseæ (Chap. XXX) is based on anatomical characters, while the alternative name Neuropterideæ is restricted to fronds. Attention is called to the great length of the Medullosean stems, specimens of *M. stellata*, for example, reaching a length of nearly 8 metres, and of 3½ metres without a leaf or branch. It is suggested that these long stems with scattered leaves may have had a climbing habit, and a comparison is made with the anatomy of certain tropical lianes. An excellent comparative account of the structure of the stem is given, with a useful set of diagrams (p. 92) and there is a striking quotation from White about the "amazing engineering experiments" which Nature was trying in this group in Palæozoic times.

The author considers that there is little or no doubt that the fronds of *Medullosa anglica* bore seeds of the genus *Trigonocarpus*. The seeds discovered by Dr. Kidston in connection with leaflets of *Neuropteris heterophylla* and *obliqua* (by Kidston and Jongmans) are regarded as showing a close affinity to *Trigonocarpus*.

The subject of the male organs, about which our information is still scanty, is fully considered. *Potoniea* and *Codonothea* are cited as probable microsporangiate fructifications of this group, while the problematic *Whittleseya*, formerly referred to the Ginkgoales, is compared with *Dolerophyllum*, both being probably microspore-bearing leaflets of Pteridosperms, possibly Medulloseæ.

Colpoxylon, with from 1 to 7 steles, is maintained as a distinct genus. The new figures of this stem are welcome. The imperfectly known fossil *Rhexoxylon africanum* is described, in accordance with Miss Bancroft's results, as a polystelic stem related to the Medulloseæ.

In connection with *Sutcliffia*, Dr. Ethel de Fraine's suggestion of the two courses of evolution which may have started from this type—a monostelic line leading to the Cycadophyta, with a polystelic branch to the more complex Medulloseæ—is discussed. The author evidently inclines to this view, while giving full weight to the arguments of Worsdell and Matte in support of a polystelic ancestry of the Cycads. The reviewer is quite in sympathy with Miss de Fraine's hypothesis, but feels that connecting links between *Sutcliffia* and the Cycad type are badly wanted.

¹ On p. 81, *Sphenopteris obtusifolia* is a misprint for *S. obtusiloba*.

The polystelic stem *Steloxylon* has long puzzled palæobotanists; the latest view is that of P. Bertrand, who thinks that the plant is a form of *Cladoxylon*. It is a pity that the transverse section of the stem is not figured.

Chap. XXXI is devoted to the seed-bearing fronds of Pteridosperms such as *Pecopteris Pluckenetii* and *Aneimites* (here called *Wardia*) *fertilis*, where only external characters are known.

The author attributes the winged appearance of the seeds in *P. Pluckenetii* to the presence of a sarcotesta, and suggests an affinity with Medulloseæ rather than Lyginopterideæ; the same applies to *Wardia fertilis*. The seed *Samaropsis acuta* is believed to belong to an *Eremopteris*, as first suggested by Duns as long ago as 1872, while Nathorst's *Lagenospermum Arberi* is associated with an *Adiantites* from Spitzbergen (Lower Carboniferous).

Chap. XXXII is headed Cycadofilices; the distinction from Pteridosperms is clearly arbitrary, though unavoidable while our evidence is so scanty as to the fructifications. It may be asked however why *Steloxylon* should be placed in Pteridospermeæ and the almost identical *Cladoxylon* in Cycadofilices.

It is remarkable how many of the families of Cycadofilices—Megaloxyleæ, Rhetinangieæ, Stenomyeleæ and Protopytyæ¹—are monotypic. Rhetinangieæ might perhaps be merged in Lyginopterideæ, but the others are certainly isolated types. Our knowledge of the whole group must evidently be very fragmentary; possibly we may be dealing with the survivors of families which had their day in an earlier period.

In the account of the Cycadoxyleæ the Cycadean features might perhaps have been more emphasized. These plants seem to be the nearest approach we have to connecting links between Pteridosperms and Cycadophyta.

Zalessky's separation of *Eristophyton* from *Calamopitys* is adopted, but the new genus is included in the Calamopityæ—a reasonable compromise between opposing views.

Two chapters are devoted to the Cordaitales, the great class of Palæozoic Gymnosperms. The family Poroxyleæ is taken first, and the description is illustrated by photographs supplied by Prof. C. E. Bertrand. The habit of the plants is compared with that of Bamboos. In describing the Cordaiteæ the name *Cordaites* is still used in a comprehensive sense, while special genera such as *Mesoxylon* are wisely restricted to anatomical specimens. The genus *Araucarioxylon* is rejected, and all wood of the general Araucarian type which cannot be more definitely determined is included under *Dadoxylon*.

Stress is laid on the wide distribution of the Cordaiteæ (including *Næggerathiopsis*) which spread practically all over the world. As regards range in time, while Cordaitales no doubt existed in pre-Carboniferous Floras, there is no evidence that the *Cordaites* type was then represented. At the other end of the scale, it is probable that the family Cordaiteæ came down at least to the Rhætic.

The Cordaitan structure is described as xerophilous, and it is suggested that the frequent absence or slight development of palisade tissue in the leaf may point to diffused rather than brilliant sunlight.

¹ P. 212, line 9; the statement refers to the *primary*, not to "the inner part of the secondary xylem."

The structure of the flowers (*Cordaianthus*), so familiar from Renault's drawings, is now illustrated by photographs of Prof. Bertrand's, which are interesting if not always clear.

In the next chapter (XXXIV) the family Pityeæ is described. A note by Dr. Gordon, referring to his unpublished observations on *Pitys*, will be read with much interest. He finds medullary as well as circum-medullary xylem-strands in *P. primæva* and *antiqua*, and also in his new species, *P. Dayi*. A closer connection with the Kentucky genus *Archæopitys* is thus established. In *P. Dayi* the leaves and leaf-traces have been investigated. The leaves are quite different from those of *Cordaites* and their structure "tends to accentuate the possible relationship of *Pitys* with the *Lyginodendrea*" (p. 288).

While Zalesky's Devonian genus *Callixylon* undoubtedly finds its right place in the Pityeæ, the inclusion of other genera, such as *Parapitys*, *Cænoxylon*, and *Mesopitys*¹ is more open to discussion. *Antarcticoxylon*, possibly allied to *Mesopitys*, is of interest from the high Southern latitude (74° S) at which it was found.

Chap. XXXV is concerned with such Palæozoic Gymnospermous seeds (the great majority) as cannot be definitely assigned to their parent plants. The main divisions Radiospermeæ and Platyspermeæ are not adopted, and the seeds are grouped in three classes, Lagenostomales, Trigonocarpales and Cardiocarpales, the two former being essentially radiospermic, and the last platyspermic. The subject of Palæozoic seeds is as difficult as it is important, and students will find the author's full synopsis of the greatest service.

In Chap. XXXVI we come to the great class Cycadophyta, the dominant Seed Plants of the Mesozoic. The chief division is of course that of the Bennettiales, including *Williamsonia*; Chap. XXXVI deals with the Bennettiales proper, and the following chapter with the "Williamsonian tribe," to use Wieland's phrase. The author, "with some reluctance" gives up the use of Carruthers's name *Bennettites* for the genus, and adopts Buckland's *Cycadeoidea*, which has the priority and has been constantly employed by the American investigators in their classical work. Dr. Marie Stopes's attempt to discriminate between Buckland's *Cycadeoidea* and *Bennettites* by structural characters is criticised, and her conclusions regarded as insufficiently established.

The complex organisation of *Cycadeoidea* is fully described. The author suggests a doubt whether the megasporophylls in some of Wieland's bisexual flowers, were "merely immature or functionless as in the male flower of *Welwitschia*" (p. 379). The former had generally been assumed, but the question raised calls attention to the need for a fuller investigation of the ovular stage of the gynæceum. There are some further data in Wieland's second volume, which came too late for the author to make use of it.

The significance of this wonderful group of plants is well expressed in a quotation from Lester Ward: "Cycads are to the vegetable kingdom what Dinosaurs are to the animal, each representing the culmination in Mesozoic times of the ruling Dynasties in the life of their age" (p. 385).

¹ On p. 294 *Mesopitys* is inadvertently included among genera with a double leaf-trace, cf. p. 296.

In the detailed account of *Cycadeoidea* (*Bennettites*) *Gibsoniana*, *phloem* is doubtless a *lapsus calami* for *periderm* (p. 388, line 16).; internal phloem would be a remarkable feature in a Bennettitean stem.

The author is sceptical as to the flower of *C. Gibsoniana* having been bisexual, for he finds no trace of the stamens (p. 395). Considering the proved bisexual character of closely similar American specimens, it seems most probable that the disappearance of the stamens is merely due to the maturity of the fruit, but here again Wieland's later work must be consulted.

On the vexed question of the morphology of the Bennettitean gynæceum the author takes the view that the seed-stalks (megasporophylls) and scales are homologous, being respectively fertile and sterile carpels, enclosed by a perianth of bracts (p. 403).

Vectia, a large mass of fossilised phloem, discovered by Dr. Stopes at Luccomb Chine, is referred on good grounds to the Bennettitales, rather than to the Coniferæ.

Carruthers instituted a special tribe *Williamsoniæ*, and it is probable that we shall have to return to this course, though in the meantime it may be difficult to draw the line between the *Williamsonian* and *Bennettitean* tribes.

Much attention is given to the difficult interpretation of the original specimens of *Williamsonia gigas*. There is room for some doubt as to the details, but the fact that *Williamsonia* flowers and *Zamites* leaves were borne on the same stem, confirming Williamson's well-known restoration, is fully established (see Fig. 541). The author maintains that the flowers of *W. gigas* and other species were bisexual, and that the funnel-shaped appendage believed to have surmounted the top of the gynæceum was identical with a whorl of connate stamens. The complete flower, on this view, must have been, as Mr. Thomas puts it, top-heavy, for the staminate whorls were 3 or 4 inches across, or more when expanded. A more serious objection is that in all flowers of Bennettitales which are known to have been bisexual, the staminate whorl was hypogynous, below and exterior to the gynæceum. This is the case in *Cycadeoidea*, *Cycadella*, *Wielandiella* and *Williamsoniella*. On Prof. Seward's view, precisely the opposite arrangement prevailed in *Williamsonia*. The analogy with the hypogynous and epigynous flowers of *Ericaceæ* (p. 458) is scarcely adequate, for even in an epigynous flower the gynæceum is central, whereas on this hypothesis the central organ of the flower in the Bennettitales would have been sometimes the gynæceum, sometimes the andræcium. The comparison with *Williamsoniella*, so admirably investigated by Mr. Thomas, seems specially unfavourable to the author's view, for here the agreement with *Williamsonia* is close, extending even to the presence of a "corona" at the apex of the female receptacle, and yet the staminate whorl is hypogynous. In this remarkable family all things are possible, but a good deal more evidence will be required before the author's daring hypothesis can be accepted.

Apart from controversial points, the whole account of the *Williamsonia* group is most interesting, and gives a striking idea of the wide range of organisation in the Bennettitales. Some of

the plants described, such as *Wielandiella* and *Williamsoniella*, must have been totally different in habit from anything which we associate with Cycads.

The two concluding chapters contain an account of stems and fructifications other than those clearly referable to Bennettitales, and of Cycadophytic fronds generally. It is remarkable how scanty is the evidence for the occurrence of true Cycadaceæ in the Mesozoic Floras, though it seems to be sufficiently proved that they were present.

The Bibliography extends to Vol. IV as well as Vol. III. When the book is completed a single Bibliography for the whole would be convenient.

The concluding volume will deal with the remaining Gymnosperms—Ginkgoales and Conifers—and we may no doubt hope for some further discussion of the general results of palæobotanical research.

The volume which we have briefly considered not only shows the author's unrivalled acquaintance with the literature of his subject, but is at the same time a record of extensive personal researches. In addition to his important original work, the author has re-investigated a number of the fossils for the purposes of this work, and has often illustrated their structure by new figures. The volume is thus of equal value to the investigator and to the University student.

D. H. S.

ON SOME CRITICISMS OF THE OSAZONE METHOD
OF DETECTING SUGARS IN PLANT TISSUES.

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SINCE the publication of two papers² dealing with the use of phenylhydrazine for locating sugars in plant tissues some criticisms of certain points dealt with have appeared, and have been quoted. As these criticisms seem to be based in part upon a somewhat inadequate appreciation of the significance of the observations recorded, I propose to attempt to emphasise here some points which I had hoped would have been clear to readers of the papers in question.

In the interesting account by Davis, Daish and Sawyer³ of their work on the carbohydrates of the mangold leaf a note appears⁴ from which the following is extracted:

"It is difficult to understand Mangham's view that it is possible to discriminate between dextrose and lævulose by means of the osazone test, seeing that both sugars (as well as mannose) yield identically the same osazone: Mangham seems to regard the osazones from dextrose and lævulose as distinct substances.

"In the writers' opinion little reliance can be placed on a micro-chemical osazone test as a means of identifying maltose in plant tissues, owing to the presence of large quantities of other sugars. Our quantitative analyses (some 500 in all) have in no single instance disclosed the presence of even traces of maltose in the leaves or conducting systems of plants. In work of this kind micro-chemical tests as a means of distinguishing individual sugars should be avoided and only quantitative methods adopted. Otherwise contradictory and uncertain results are inevitable."

In their review of recent work on carbon assimilation Jörgensen and Stiles⁵ make reference to the above criticisms, and add:

¹ Baeteriologist (Temporary), Naval Medical School, Royal Naval College, Greenwich.

² Mangham, S., 1911. "On the Detection of Maltose in the Tissues of certain Angiosperms." *NEW PHYT.* **10**, pp. 160-166.

Mangham, 1915 "Observations on the Osazone method of locating sugars in Plant Tissues. *Ann. of Bot.*, XXIX, pp. 369-391.

³ Davis, W. A., Daish, A. J., and Sawyer, G. C., 1916. "Studies of the Formation and Translocation of Carbohydrates in Plants. I. The Carbohydrates of the Mangold Leaf." *Journ. Agric. Sci.*, VII, pp. 255-326.

⁴ *l.c.*, p. 311.

⁵ Jörgensen, I., and Stiles, W., 1917. "Carbon Assimilation," *NEW PHYT.* Reprint, No. 10, p. 128.

"The fact that Mangham should claim to distinguish between *d*-glucose and *d*-fructose in the plant by means of the osazone test, when their phenyl osazones are of course identical, is not very reassuring as to the degree of reliability of his results."

It is assumed by the above writers that in the papers dealt with, a *chemical* difference is considered to exist between the osazone yielded by dextrose and that yielded by *lævulose*. Surely this is somewhat gratuitous! It really looks as if sufficient attention could not have been given to that section of the later paper headed "Effects of Glycerine," a section occupying some fourteen pages. A few extracts may perhaps serve to make this clear.

"The use of glycerine in the reagent has several advantages. . . . On the other hand it has some effect upon the reaction with sugars, a point which does not appear to have received adequate attention at the hands of other botanical workers or critics who have dealt with it."¹

" . . . it has been found that the glycerine tends to hinder or prevent crystal formation to an extent which varies with the different sugars."²

"This effect appears to be more pronounced with maltose and dextrose than with levulose."³

A number of experiments were described in which the effect of glycerine was observed upon the production of osazones from sugars present in various concentrations. In the case of 1% mixtures the following result was recorded.⁴

"The osazone came down more rapidly with levulose than with dextrose [this difference was observed by Senft], and in the former consisted of sheaves of long, fine, acicular crystals which contrasted strongly with the more spherical and somewhat feathery clusters of smaller though acicular crystals given by dextrose."

The actual extent of the "claim" to distinguish between dextrose and levulose by the use of Senft's reagent may be gathered from the footnote occurring on the same page:

"It may be remarked that these two types of crystal clusters can hardly be regarded as altogether distinctive characters for dextrose and levulose. In low concentrations the difference is less pronounced and may quite disappear."

Similarly in the summary:

"Too much reliance should not be placed on the crystal cluster form as a feature distinguishing dextrose from levulose."⁵

¹ *l.c.*, p. 371. ² *l.c.*, p. 372. ³ *l.c.*, p. 374. ⁴ *l.c.*, p. 376. ⁵ *l.c.*, p. 388.

The effect of glycerine on the re-crystallisation of osazones prepared from the various sugars used was also studied, the osazones being dissolved in the glycerine by heating. It was found that while some irregularities occurred, yet ". . . crystallisation seems to be retarded by the viscosity of the glycerine. Naturally in a viscous medium the rate of diffusion of particles to form crystals is slower than in a medium such as water."¹

In the section entitled "Reaction with Mixtures of Sugars," attention was directed to the work of Scherman and Williams who, using various concentrations of sugars, found that levulose, in aqueous solution, precipitated its phenyl osazone in about one-third of the time required by the same concentration of dextrose.²

The point of all this is that *d*-glucose and *d*-fructose with phenylhydrazine yield osazones of the same chemical composition, but that this osazone is formed in the one case from an aldose and in the other from a ketose; the results of the experiments of Senft, Sherman and Williams, and myself indicate that the formation of the osazone occurs more rapidly from *d*-fructose than from *d*-glucose in aqueous solutions, while in strong glycerine the viscosity of the medium retards the precipitation in both cases, and to some extent also affects the configuration of the resulting crystal clusters of the osazone, as shown by various figures and photographs in the papers under consideration. It is then a question of the influence of viscosity upon molecular rearrangements, diffusion and crystal formation.

With regard to the use of Senft's reagent for identifying maltose, difficulties undoubtedly exist, but here again they are due mainly to the influence of the glycerine; this greatly retards, or may completely prevent the production of crystals from the familiar syrupy condition of the osazone.

This effect of glycerine was studied both by producing the osazone from maltose in the presence of excess of strong glycerine, and by observing the re-crystallisation of the osazone from glycerine in which it had been heated.³

As the result of the experiments performed the following opinions were expressed:

"Considerable caution must therefore be exercised in attempting to locate maltose in starch-forming plants by means of Senft's reagent. The formation of drops of syrupy liquid within cells, especially if in any quantity, and if in tissues examined after starch dissolution is known to have occurred, in all probability denotes

¹ *l.c.*, p. 381.

² *l.c.*, 385-6.

³ *l.c.*, pp. 378 and 381.

the presence of maltose, though other possibilities are not altogether excluded.

"If actual crystals are formed the osazone can be identified with less uncertainty, though here again it is necessary to bear in mind alternative interpretations."¹

In this connection the work of Daish² on the occurrence of maltase in plants is of interest. This enzyme was found to be present almost universally in starch-forming plants, and to be abundant in the leaves. Maltose, however, was not found by Davis, Daish and Sawyer³ in the leaves or conducting systems of plants, and its absence was considered to be due to ". . . the amount of maltase always being in relative excess in the cells where the starch degradation actually occurs, so that it is able to deal instantly with the whole of the maltose formed from the starch. The fact that maltose, unlike cane sugar, never occurs in the stalks or conducting vessels is probably due to the fact that maltase is an intracellular enzyme and apparently acts in close collaboration and in the immediate proximity of the ordinary diastase which first attacks the starch in the cells where this substance is stored."⁴

There is considerable interest in the above suggestion, although some time may yet elapse before the distribution of intracellular enzymes within the cell can be determined with any degree of reliability.

It can readily be understood that an excess of maltase within cells containing starch undergoing hydrolysis might prevent any accumulation of maltose. But it can scarcely be that maltose is completely absent from the cells at all times, for it must come into existence for the maltase to act upon it, and until it can be *proved* that the maltose is produced in actual contact with the maltase it is reasonable to suppose that this sugar persists in the cell, at least for the short time required for it to travel to the enzyme prior to hydrolysis.

The ordinary equilibrium relations obtaining in enzyme actions would presumably make the *complete* destruction of maltose within the cell unlikely while any dextrose remained in the same cell. It is, however, quite conceivable that owing to the activity and abundance of the enzyme, although the *total turnover* of maltose

¹ *l.c.*, p. 379.

² Daish, A. J., 1916. "The Distribution of Maltase in Plants." *Biochem. Journ.*, X, pp. 31-76.

³ *l.c.*, pp. 311, 313, 353, 354, 357, 358 etc.

⁴ *l.c.*, p. 358.

might be great, yet at any one moment this sugar might be present only in such small amount as to escape detection even by very careful extraction methods.

In the course of my own work—not primarily directed towards the determination of sugar changes in leaves—no evidence for the presence of maltose in anything but very small total amounts has been found. What has been regarded as in all probability maltose phenylosazone has been seen as a rule in small numbers of isolated parenchymatous cells of leaf veins, and in the sieve-tubes of the veins, for the most part the finest veins.

I can well believe that such amounts could easily escape detection by methods involving extraction of leaf pulp, but I feel as the result of spending much time in observing many sections of plant tissue after treatment with Senft's reagent, and in examining controls with pure sugars, that often maltose really can be detected in this way. I have, however, never been able to demonstrate its occurrence in anything in the least like the amount in which hexoses occur. Indeed, the scarcity of maltose was formerly the cause of considerable surprise in view of the results described by Brown and Morris.

It should be almost unnecessary to insist that my papers on the osazone method dealt with the results of work by means of which it was sought to ascertain the degree of reliability of Senft's reagent for diagnostic purposes.

This work has shown that by the use of Senft's reagent alone it is not possible to distinguish with certainty the individual sugars of a mixture, still less to determine the proportions in which they are present.

While the reagent fails to furnish an ideal microchemical sugar test suitable both for qualitative and quantitative work, yet its use in certain forms of research is of considerable value, provided that its limits are always kept in mind, and that so far as conclusions with regard to individual sugars are concerned the results yielded are regarded as indicating probabilities rather than as affording demonstrations.

At the present stage of inquiry into the nature of the changes undergone by carbohydrates in foliage leaves, etc., it would be as well to try to get the most out of all available means of attacking the problems met with, in the hope that eventually sufficient evidence may be accumulated and collated to enable conclusions to be drawn with less uncertainty than is as yet possible, in spite of the vast amount of laborious work which has been carried out.

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THE RECONSTRUCTION
OF
ELEMENTARY BOTANICAL TEACHING.

A DISQUIETING feeling that all is not well with the position of academic botany in this country has been growing for several years in the minds of many botanists. The present memorandum is an attempt to analyse the grounds of this feeling, to define as far as possible what seems to be wrong and to seek a means of escape from the existing position, which is not only unsatisfactory, but even dangerous.

When the subject is discussed among those who share the dissatisfaction referred to, different points of view, varying according to the predilections, temperaments, and experience of the speakers, are naturally reflected. But there seems to be this in common between those who are dissatisfied with existing conditions—a general impression of lack of quality and of vitality. Some attribute this lack to the diverse nature and wide range of the actual methods employed in the study of plants. The content of modern botany has become so vast that the student's attention and energy are dissipated and he gets very little thorough knowledge of anything. On the other hand it has been held that one of the great virtues of the subject lies in these very qualities of variety and extent, which provide freshness of interest and breadth of outlook. The actual effects on the student of these qualities of modern botany are, however, conditioned by the manner in which the subject is presented to him, and it is widely felt that an excessive amount of time is devoted to teaching and learning the less vital parts of the subject. Other critics of the existing state of things attribute the lack of vitality and originality to the poor mental quality of the students themselves, others again to the very poor remuneration available to any save a few botanists and to the

paucity of reasonably paid research posts, and yet others to the limited outlets on practical life which botany at present affords.

Doubtless all these factors are involved, and many of them are certainly largely related as cause and effect. Thus if a great deal of the actual work done in the subject, and especially that to which the student's attention is largely directed from the outset, is not of a nature or quality to attract and interest the best types of mind among possible students, the average quality of student is bound to be low. If the ultimate income to be expected in any career to which the study of botany leads, except for a few reasonably well paid posts, is absurdly low—much too low to satisfy the reasonable demands on life of the average vigorous-minded man of normal desires, and if the outlets on practical life are few and narrow, vigorous-minded men will be few among botanical students. This state of things will react on the quality of the teachers, who are drawn from among the students of a few years ago, and who will also be without the stimulus afforded by first-rate and active-minded pupils. The whole set of conditions becomes a vicious circle, which has to be broken at some point if there is to be any real and general improvement. Where is it practicable to break the circle? The view taken in this memorandum is that one point which it is essential to attack and which can be successfully attacked is the reform of elementary teaching.

Botany in this country is still largely dominated by the morphological tradition, founded on the attempt to trace the phylogenetic relationships of plants, which began as the result of the general acceptance of the doctrine of descent. Elementary teaching (as well as a very large part of advanced teaching) is mainly occupied with the endless facts of structure and with their interpretation from the phylogenetic standpoint. Side by side with this there generally goes a discussion of function which is often limited by a crude Darwinian teleology. Plant physiology is relegated in most cases to a subordinate place and is taught as a separate subject. The newer studies of ecology and of genetics play a very small part in the curriculum. The result is that the student's introduction to the study of plant life is unbalanced and has a definite morphological bias. He inevitably comes to regard the most vital parts of the subject—those dealing with the plant as a living organism—as specialised studies of subordinate importance. The elementary student is not clearly shown the essential basic importance of these studies, which should be fundamental, because his teaching is mainly in the hands of men who are primarily morphologists.

This state of things reacts to the detriment of the teaching of these more vital studies themselves. The teaching of physiology in particular suffers from its sharp isolation from the general study of plants, and tends to become excessively academic—often a somewhat dreary catalogue of the results of inconclusive experiments, and in the laboratory a series of exercises in test tube reactions and in the use of standard pieces of apparatus. It is inevitable, of course, that much advanced work in plant physiology should be specialised work in what is now called biochemistry and biophysics, dealing, as it were, only incidentally with plants themselves. The elementary teaching in plant physiology generally current is not even a good introduction to that. What is wanted is something broader, more vital and more practical, something which shall at one and the same time vitalise the student's first introduction to the study of plants, exhibit its connection with practical life, and serve as an introduction to the specialised advanced work. We must put an end to the divorce of elementary training in the study of function from the general study of plant life, a divorce which will necessarily continue while general courses in elementary botany are planned primarily on morphological lines.

It is not denied that morphological botany in the hands of a teacher of personality and vigour can be made interesting and a real instrument of education. The same is true of almost any branch of knowledge. What is maintained is that morphological botany *ought not* to be made the main topic of elementary education in botany, because, in its current form at least, it is sterile and leads to little but further refinements of itself, and because it has no outlets on practical life. Morphological botany does not attract the type of mind that wants a deeper insight into the working of plant processes or into the part which plants play or can be made to play in the economy of the world, and it does give an unfortunate bias to the type of mind whose activities can be turned with equal success to the tracing out of an obscure phylogeny or to the solution of a problem in ecology or biochemistry.

Various efforts which have been made to separate plant physiology from morphology bear upon the point under discussion. It is noteworthy that these efforts have never come from morphologists but always from physiologists. More than 20 years ago certain animal physiologists, struck by the close bearing of some of the recent work on carbohydrate metabolism in plants upon similar problems in animals, desired to combine plant physiology and

animal physiology in one section of the British Association. This suggestion was, however, vigorously opposed by many botanists, who contended that the study of plants must be maintained as one subject. Their contention was successful and the new section was founded for animal physiology alone. Quite recently a movement, this time initiated by plant physiologists, was set on foot at the University of London to separate plant physiology from morphology in the honours degree examinations of the University, so that a student could take his degree in either branch. This movement was the outcome of the enormous increase in the content of the curriculum of advanced botany, which was felt to impose an unnecessary and too burdensome a strain on students whose interests were primarily physiological. The proposal met with considerable support, but was not carried further at the time owing to the outbreak of the war.

It is inevitable that separation of advanced work on some such lines will occur sooner or later. It is already impossible for the student adequately to cover anything like the whole of the ground represented by modern botany, and some measure of relief cannot much longer be refused. If such a splitting as was contemplated were carried into effect, the present elementary courses would at first no doubt be retained as an introduction to the specialised advanced work, on both morphological and physiological sides, but it would soon be found that the existing courses were both inadequate and redundant as an elementary training for the plant physiologist. A movement would then probably be set on foot to provide a better training in chemistry and physics for the budding plant physiologist and to dispense with the unnecessary and unsuitable "elementary botany." If this anticipation of the course of events is correct, the separation of the old subject of botany would then be complete. At this point it may be asked: Why not leave events to take their course? If the tendencies at work lead to such a development, why try to deflect them into some other channel? If plant physiology will thus eventually free itself from the shackles of morphology why not let the thing happen in that way? The answer is: Because it would be bad for the subject and bad in various degrees for everyone concerned. This conviction is based not on a mere sentimental desire to maintain the study of plants as a single subject, but on the belief that you cannot divorce specialised work connected with plants from a sound elementary training in the study of plant life as a whole without narrowing and formalising

the specialised work. It is no doubt true that every problem in plant physiology is ultimately a problem in chemistry or physics, but it is equally true that the biochemist and biophysicist can only acquire the perspective in which his problems may be properly chosen and posed if he has had a broad general training in elementary botany as well as in chemistry and physics. Though such a training is supposed to be available in all Universities, in most cases this is not the fact because the elementary teaching is dominated by morphology.

The evil effects of the formal divorce of physiology from morphology are strikingly illustrated in the sister science of zoology. As a result of the independent development of animal physiology in relation to medicine, zoology has largely become synonymous with comparative anatomy, and has sunk into a condition of sterility in which it no longer attracts the student. Attempts to revivify zoology by laying stress on the field study of animals, on economic entomology or on genetics—though these studies have had brilliant results in their special fields—have not been successful in lifting the subject as a whole from the ruts into which it had fallen, precisely because such attempts do not envisage the study of animals as a living whole, and this cannot be done if animal physiology as the essential basis of treatment has to be left out of the account. Botany is threatened with a like fate if botanists do not quickly set their house in order. It has not yet reached the stage of formal segregation, but it has become a concatenation of specialised subjects which have lost organic connexion, and the formal separation will inevitably follow unless the subject can be given an organic unity by insistence on vital elements.

The case of those who become pure morphologists need not detain us long. There will always be some minds which find their satisfaction in tracing out formal relationships, though it is significant that more and more botanists who have been brought up in that tradition are abandoning it for other lines of work. Certainly those who eventually devote themselves to pure morphology cannot but benefit by acquiring at the outset of their careers a deeper insight into the plant as a living organism. Indeed it is only from such a deeper insight that any escape of morphology from the mere phylogeny tracing, which has become too prevalent, can be expected.

It is not necessary, and it would be grossly unfair, to belittle the actual achievements of those leaders of research who have devoted themselves with brilliant results to the investigation of phylogenetic

problems. The British school of morphologists, whose work largely derived its inspiration and opportunity from the revival of the study of fossil plants some 20 or 30 years ago, has won a distinguished reputation throughout the world. It has worthily upheld the loftiest traditions of pure science and it has inspired in many younger workers a profound interest in their subject and an intense devotion to those traditions. The very distinction and success of the school has indeed tended to overshadow academic botany in this country, and to divert effort and enthusiasm from the channels which we cannot but regard as most essential to the healthy life of botany as a whole. Nowhere has this effect been more marked than in the sphere of elementary teaching.

But between pure morphology, on the one hand, and physiology in its purely chemical and physical aspects, on the other, come a number of lines of activity which seem to promise great developments in the immediate future and to have numerous outlets on practical life. The middle strata, so to speak, of botanical knowledge and investigation, consisting of problems and topics which cannot yet be subjected to the deepest chemico-physical analysis, but which really form, or should form, the very core of the study of plants as living organisms, are precisely those which are neglected by teachers who are either pure morphologists or academic physiologists—men who have an intimate acquaintance with structure or who can use plants as the vehicles of chemical and physical experiments, but have no practical first-hand knowledge of the life of plants in the field or in the garden, men in fact who are remote from reality in all that concerns plants as living beings.

A drastically reconstructed course in elementary botany seems to be the essential starting point of an adequate training for these lines of work. Elementary teaching should be founded in the first place on a thorough recognition of the fact that the fundamental structure and activities of plants and the part they play in relation to the inorganic world and to animals, are conditioned by their chemical and physical nature. This may seem a platitude, but how far the average University teacher is from bringing home to his students any real recognition of this basic principle may be readily learned from the answers to examination questions. Then there should be a treatment of the physiological as well as the structural life history of the individual plant, passing on to different ecological types with their physiological as well as their structural characters, and leading to an elementary treatment of competition and the

social life of plants. The field of ecology should thus be developed on a physiological basis. The whole of the range of topics briefly indicated here have direct bearings upon practical life, and no opportunity to illustrate these should be missed. Here we have the scientific bases of agriculture, of forestry, of the economic utilisation of waste lands, of the use of plants in coast protection, of every industry in which man grows plants, or employs plants which grow spontaneously, for specific purposes, for his own use or for the use of his animals. The scientific bases of these practical pursuits are at present left to the instruction of institutions of applied botany such as the schools of agriculture and forestry, which are not well adapted for such elementary and general instruction. The foundations should be laid earlier in the students' course and more broadly, and it is the business of botanists to lay them, because the roots of the scientific development of all these practical activities must lie in the scientific study of plants.

Then we have genetics and its practical outlet in plant breeding. In one sense genetics may be regarded as a narrow and specialised study, but it is pre-eminently alive and concerned with life, and one of the duties of an elementary teacher of botany is to see that his students are properly instructed in its rudiments and understand its relation to the general body of biological knowledge, as for instance the relation of the development of inherited characters to the environment. Such an understanding would go far to widen the outlook of the plant-breeder and to save him from certain rather conspicuous defects which are apt to characterise his work.

In such an elementary course as is here contemplated, comparative morphology would have its place, but necessarily a far more restricted and less conspicuous place than it holds at present. It seems entirely out of proportion to go through each group of plants in detail and to enter into the minutiae of their structure and relationships while an immense number of the vital problems of plant life are neglected altogether. A brief but comprehensive survey of the plant kingdom would be required in order to illustrate the principle of the division of labour and the gradual adaptation of plants to land life. Morphological methods could be illustrated in detail from a few selected instances. Considerations of time and of proportion would necessitate a rigid selection of the available material. It has to be frankly recognised that the study of the detailed evolution of the plant world, which has acquired a factitious importance owing to the overwhelming effect on the

imagination of botanists of the doctrine of descent, has no valid claim to the dominating position which, especially in this country, it has so long held.

It would be out of place in this memorandum to discuss in any detail the schools of pure botany that should be available to the student who has taken the elementary course and wants to go on with the subject. Broadly speaking, there would be three lines of natural development, two of them with broad outlets on practical life. First the more generalised pursuit of plant physiology and ecology, which would centre in the study of plant life and its relations to environment; secondly, the specialised biochemistry and biophysics of plants; and thirdly, advanced morphology, including so-called "systematic botany." The first would naturally lead, on the practical side, to agriculture and land utilisation in the widest sense, the second to the numberless detailed problems concerned with the physico-chemical reactions of plants and plant substances in agriculture and in industry. In each the student should have, according to his individual aptitudes and inclinations, an opportunity of entering upon research, either on the purely theoretical or on the practical applied side. From the one he might, if he chose the latter course, go on to a school of agriculture or forestry, from the other he might go to a technological institute or take a research post in an industrial undertaking. In any case he would have had a training which would fit him better than any training now available for turning his powers and knowledge to good practical use.

The establishment of a firm connexion with practical life is regarded as absolutely vital to the scheme, and this for two reasons. In the first place, broad outlets on practical life are essential if we are to tap the human material without which botany will never develop its full strength. Secondly, and even more important, if that be possible, the human mind is so constituted that in the great majority of cases intellectual activity divorced from practical life tends to what is called "academicism," whose characteristic vices are formalism, pedantry, and hair-splitting. It is true, of course, that the pursuit of knowledge for the sake of knowledge and without reference to practical utility is one of the functions in which the human mind may rise to its greatest height. But only minds of the finest and rarest quality are capable of prolonged activity of this sort without falling into the vices of academicism. The human mind, like the human organism as a whole, is primarily and fundamentally a mechanism for getting things done, not for abstract

thinking and the pursuit of knowledge for its own sake, and the more closely intellectual activity is related to action the more intimately the deep-lying instinct for *doing* is satisfied. In this psychological fact lies the essence of the real case for scientific as distinct from literary culture, because science—especially experimental science—increases our power of doing things and gives a new outlook on the whole world of active life. When the pursuit of science is divorced from life it tends to sterilise the average mind, not because it ceases to be science, but because it gives more and more the feeling of unreality.

Further, and this is also of great importance, practical life offers for solution a large number of problems which actually interest the greatest number of workers, and the continual inflow of these constantly vitalises the atmosphere of the laboratory and stimulates the interest and activity of the workers.

In devising a scheme of scientific training we have to keep these considerations constantly in mind. We have to train teachers who have a firm grip on essentials, but besides that, we should provide for the many who will soon enter upon some form of practical pursuit as a means of livelihood, for the smaller number who are fitted to do useful work in the investigation of practical problems, as well as for the few who are capable of the highest type of research without reference to immediate utility. All should have the same elementary training, in the case of botany based on the life of the plant and its relation to the life of the world at large. At each stage of higher development opportunity should be provided of branching off into practical pursuits, or into the direction of practical pursuits, till the few who are left, whether they engage in research or in teaching or in both together, will be only those best fitted to advance or to expound the theoretical knowledge without which practical results must remain sunk in empiricism. Only in the closest connexion, the constant interaction, of the spirit of science and the spirit of practical life can any branch of scientific training realise its highest ideal.

All the considerations that have been put forward were as true four years ago as they are to-day, because they are based on practical human needs and on the fundamental nature of the human mind. But the realisation of the conditions in which England, and indeed all the countries of the civilised world, will find themselves after the war multiplies tenfold the force of what has been

urged. That which was very desirable before the war has now become an imperative necessity. If botanists are to do their share in the struggle to re-establish normal life and to secure the increased vitality and efficiency which will be so evidently necessary if we are to maintain ourselves under the new conditions the demand must be met and met at once. All kinds of practical problems, certainly not least those relating to plant life and plant utilization, will press for solution with an intensity hitherto unknown. The new generation will insist on a greater reality in life and in the pursuit of knowledge with a wholly fresh urgency. Ours is a country whose governing class is singularly devoid of scientific culture, and whose national virtues certainly do not include any excessive respect for science or for theoretical knowledge of any kind. One danger which cannot be ignored is that in the overwhelming desire for quick results we shall see a widespread attempt to bring about a sharper separation than ever of practical training and of practical pursuits from broad scientific foundations. That way destruction lies, and the only adequate measure of safety is frankly to face the truth and whole-heartedly to set ourselves to our share in the work of reconstruction. We have to readjust our intellectual ideals as well as to reconstruct the material fabric of civilisation on sounder lines. Many changes are necessary, a different curriculum and a different attitude towards knowledge and science in the schools, a new organisation of research, an immense increase in its endowment, and at the same time a larger supply of better human material for scientific work. Elementary scientific training at the Universities is the link between these two fields, the schools on the one hand and research on the other, and it is the business, the immediate business, of academic botanists to see that so far as they are concerned that link is made sound and capable of properly fulfilling its function. It is of no use to tinker with the situation. It is of no use to say: "Oh, yes, by all means let us introduce more physiology into the courses." What is wanted is not patching, but reconstruction, a new spirit and a new ideal.

The immediate object of this memorandum is to obtain a basis of agreement as to what should actually be done, and the following concrete proposals are put forward as a basis for discussion.

1. The elementary courses in Botany corresponding with

the "Intermediate Science" and "Pass B.Sc." courses at most Universities, with Part I of the "Natural Sciences Tripos" at Cambridge and the first Science School at Oxford should be reorganised as suggested, *i.e.*, they should be based on a physiological treatment of the plant and its life-history, using the word "physiological" in the widest sense. To this should be added an elementary treatment of the structural and physiological characters of different ecological types with an introduction to the study of the social life of plants, and an elementary treatment of the principles of genetics. The points of contact between botany and horticulture, agriculture, forestry, and industry should everywhere be developed and insisted on. Comparative morphology should be reduced to a subordinate position, and should be used primarily to illustrate the principle of the division of labour and the progressive ecological adaptation of the various great phyla.

2. Adequate provision should of course be made for laboratory, garden, and field work in the different subjects dealt with, and in all of these, students should do as much as possible for themselves. The laboratory course should no longer be based mainly on a detailed comparative study of the anatomy of the vegetative and reproductive organs of the different groups of plants.

3. After passing satisfactorily through such an elementary course, together with similar courses in one or two other subjects, the student should be entitled to a degree in science. The standard of attainment required in the examinations attached to the course should be raised, but at the same time credit should be given for good class and practical work in estimating the claims of the candidate.

4. For an honours degree in botany graduation in an honour school of botany should be required. It is suggested that there should be three types of school—general plant physiology and ecology, biochemistry and biophysics, and morphology. A University should have one or more of such schools according to its resources. Research, or at least a practical introduction to the method of research, should be an integral part of graduation work in the honours school as well as of post-graduate work. In the two first types of school the work, while strictly scientific and theoretical in character, should be in close touch with advanced schools of agriculture, forestry and with technological institutes.

5. A determined and general effort should be made to raise the remuneration of teachers and of the holders of purely research

posts to a reasonable living wage.

The undersigned are in general agreement with the views and suggestions put forward above, and are of opinion that the time is fully ripe for action in the direction indicated. They feel that to continue in the old paths will not only be fatal to the proper status of the study of plants in the public estimation, but will mean failure in the duty of botanists to play their part in meeting the conditions which must exist after peace is declared. They therefore invite the opinions and the eventual co-operation of their fellow botanists.

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November, 1917.

This memorandum has in the first instance been published over the signatures of a few botanists who are in substantial agreement as to the necessity of reform, and as to the main essentials to which reform should be directed. The next step indicated appears to be to invite the expression of all shades of opinion upon the subject, and especially upon the practical proposals put forward in the memorandum. The Editor of the *NEW PHYTOLOGIST* therefore cordially invites the freest expressions of opinion from all botanists, and, above all, practical suggestions as to reform. He believes that the whole matter is one of the most urgent and vital importance, and that the necessary preliminary of action is to consolidate opinion and so provide a driving force. Before opinion can be consolidated the subject must be thoroughly ventilated, and the pages of this journal offer a convenient medium for discussion. Contributors are urged to write exactly what they think on any and every aspect of the subject.

THE ORIGIN AND DEVELOPMENT OF THE COMPOSITÆ.

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CHAPTER III.

IRRITABILITY OF THE POLLEN-PRESENTATION MECHANISM.

IT is probable that physiological differentiation precedes morphological differentiation in the history of a species and causal morphology must have a physiological basis for its sound study. Preliminary investigations are, therefore, in progress on the physiology of the Compositæ, and the present chapter deals with one of the most striking effects of physiological differentiation in the family, the irritability of the styles and stamens. The history of our knowledge of irritability is summarised briefly; the known cases of irritability are given, their physiology discussed, and the phylogenetic significance of the phenomenon in the Compositæ is considered in the light of the previous phyletic suggestions made in Chapter II.

A. HISTORY.

Styles :—Our knowledge of irritable styles in the Compositæ is of comparatively recent origin. The first reference to the movement of the style when touched is that in an unsigned article (30, 1815) by Ker on *Arctotis aspera*. He also records the same phenomenon in *A. aureola* (= *A. aspera*, L.), and suggests that "the style . . . seems to consist of a substance resembling elastic gum (Caoutchouc)."

Brown (4) refers only to Ker, and Cassini (7) records that he observed the phenomenon in "quelques arctotidées," but gives no details. Müller (43) and Knuth (34) give *Arctotis aspera* "and other species." Minden (42) records *Arctotis aspera* and *A. calendulacea* (= *Cryptostemma calendulacea*, R.Br.) as irritable, and discusses the physiology of the process (see below, Section D). Juel (28) extends the phenomenon to *Arctotis stoechadifolia*, and the writer has published a preliminary, illustrated account (47) of the phenomenon in *A. aspera*.

In other families the phenomenon of movement of the style or stigma when touched seems to have been known at a much earlier period, as the facts were apparently common knowledge when Kurt Sprengel (52, 1817) wrote of the irritable stigmas of

Mimulus and *Martynia* and the movements in *Scævola*, *Leeuwenhækia* and *Stylidium*. The movement in *Mimulus* is the subject of a note by Kitchener (33) and that in *Martynia* by Harger (21), while the physiology of the process in both genera and in *Strobilanthes* is considered by Oliver (44).

Stamens.:—The earliest record of the sensitiveness of the stamens in the Compositæ is that of Alexander Camerarius (5), who, while recording the phenomenon in *Cyanus Turcicus*, etc., quotes Borelli as having made the first observation on *Jacea aromatica*, the *Cardui pratenses*, the *Cyani* and *Stæbe*. Camerarius refers to the anther tube as the "vagina," but a few years later Bose (3) uses the term "stamina," and makes a passing reference to the irritability of these organs in some Compositæ. About half a century later Conte dal Covolo (10) discussed the phenomenon in *Centaurea calcitrapoides*, and gave very good figures of his detailed dissections. Previous to Koelreuter all recorded species except *Stæbe* (Inuleæ) belonged to the Cynareæ, but that author (36) extended the list to the Cichorieæ (*Hieracium*, *Cichorium*, *Scolymus*) and the Inuleæ (*Bupthalmum maritimum* = *Odontospermum maritimum* Sch. Bip.).

After the lapse of a century during which the only reference to the movement is a passing one by Cassini (7), the physiology of irritability was the subject of various investigations and discussions by Cohn (9), Unger (54-55), Hofmeister (24), Pfeffer (45) and others (see Section D). Hildebrand (23) added *Antennaria dioica*, and various Cynareæ were added to the list of "irritables" by Müller (43), Knuth (34), Meehan (40-41), Kirchner (32) and Linsbauer (37). Meehan (40) also records a peculiar irritability of the corolla in *Centaurea mexicana*, which, however, does not seem to have been confirmed. The trigger hairs on the filaments of some Cynareæ are described and figured by Kabsch (29), Unger (55), Kny (35), Haberlandt (15) and Halsted (16). The last (18) also extended the records of irritability to the Heliantheæ (*Echinacea*, *Heliopsis*)

Although he does not mention anything in the text Lubbock (38) extended the observations to the Anthemideæ. This is clear from his figures 87-88 of the anther tube in *Chrysanthemum parthenium*, in the first the tube is closed, erect, median and straight, in the second after an insect visit the tube is oblique, retracted and with the pollen exerted. Kerner and Oliver (31) mention *Onopordon* and *Centaurea* as examples, and compare the mechanism with the

mechanical action of the keel and style in some Papilionatæ. Juel, after a preliminary account, (27), extended the observations (28) to the Astereæ, Heleniæ, Senecioneæ, Arctotideæ, and Mutisieæ in addition to the Inuleæ, Heliantheæ, Anthemideæ, Cynareæ and Cichorieæ previously noted. The writer, after two preliminary notes (47-48), extended the list of "irritables" to 149 species and varieties (49), including the Calenduleæ and ten other tribes previously noted, but excluding the Vernoniæ and Eupatorieæ. Of the species observed 64% showed irritability.

Another type of movement is described by various authors, namely a gradual and automatic contraction of the anther tube. This is mentioned (25) and figured (26) by Jacquin for *Calea aspera*. Jacquin, however, considers the inclusion of the anthers to be due to an elongation of the corolla, and this is shown in his figure. Cassini (6) considered the inclusion to be due to the withering of the stamens, but Brown (4) described it as "a vital action, and not the effect of withering or decay, which, however, speedily follows it," and considered it to be analogous to the "more evident motion in certain Cinarocephalæ." Another explanation was given by Meehan (41), who suggested that the filaments of *Helianthus* were stretched during exertion by the growth of the style against the closed, upper end of the anther tube, and that the subsequent retraction was due, not to irritability, but to the elasticity and spontaneous contraction of the filaments. Asa Gray (13-14) controverted this, and gave experiments proving that the style does not stretch the filaments, and that there is a lateral movement through 15 or 20 minutes of an arc when two adjacent filaments are touched, much as in the Cynareæ. Evans (11) has a note on a so-called unusual case of the re-exsertion of the style after the usual retraction of the style and stamens, but this is more or less usual, a typical case being *Arctotis aspera* as described by the writer (47, Fig. 1), who also found irritability of the ordinary type in a number of the Heliantheæ, the tribe of which this slow retraction of the anther tube is described as typical by Brown.

This movement, however, occurs in most tribes, and it seems probable that a confusion has occurred between the ordinary type of irritability and the retraction of the stamens due to the loss of turgor in the filaments which immediately precedes the withering of these slender structures. Thus Cassini was right if this loss of turgor is considered the first step in withering, Brown was right if the process of dying is considered to be a vital process, Meehan

is right in his explanation that the retraction is due to the elasticity of the filaments, and Gray is right when he controverts Meehan on the elongation of the filaments being due to a pressure exerted by the style. The proximate cause of the elongation of the filaments seems to be an increase in turgor (see Section D).

Irritable stamens in other families were unknown to the earlier botanists,¹ and according to Goeppert (12) the first to record the movement in *Berberis* was Linné (1775). Goeppert gives a good historical account up to his date (1828). Smith (51) was the first to locate a motor region in *Berberis*. Kurt Sprengel (52) recorded staminal movements in *Parnassia*, *Cacti*, etc.; Kitchener (33) recorded a gradual contraction and contortion of the filaments in *Achimenes*; Bessey (1), Meehan (39) and Halsted (17) made various observations on the movements of the stamens in *Portulaca* spp. Halsted also recorded experiments on *Berberis* (19-20). Tomney (53) records about 50 species of *Opuntia* which show irritability of the stamens, while Pfeffer (46) records the phenomenon in *Mahonia*, *Helianthemum* and other Cistaceæ, *Mesembryanthemum*, *Opuntia*, *Cereus*, *Sparmannia* and certain Tiliaceæ and Portulacaceæ.

B. IRRITABILITY OF THE STYLES.

Outside the Compositæ the only genus recorded as having a style which moves towards the touch is *Glossostigma* (46), but the stigmatic lobes are sensitive and close when touched in various other genera such as *Mimulus* (29, 33, 44, 46, 52), *Martynia* (Martyniaceæ) (21, 44, 46, 52), *Strobilanthes* (= *Goldfussia*, Acanthaceæ) (44, 46) and *Bignonia* (Bignoniaceæ) (46). To these may be added *Torenia*. The stigma of *Torenia Fournieri* in India shows the same form of irritability as *Mimulus*, but other species, such as *Torenia vagans*, are not sensitive, nor is *T. Fournieri* when grown under glass in this country.²

In the Compositæ the phenomenon as at present known is confined to the Arctotideæ. It occurs in *Arctotis aspera* (30, 42, 47), *A. stoechadifolia* (28) and *Cryptostemma calendulacea* (28, 42). To these may be added the *Gazania splendens* of gardens. Ker (30) gives *Arctotis aureola*, Ker Gawl, also, but this is synonymous with *A. aspera*, L. The phenomenon in *Arctotis aspera* has been described in detail by the writer elsewhere (47), but a brief *resumé* is given below in order to facilitate reference.

¹ There is a considerable literature dealing with the stamens of *Berberis*, but most of it is irrelevant in the present argument.

² For this information about *Torenia* I am indebted to the Director of the Royal Botanic Gardens, Kew.

Arctotis aspera, L.

The activities of the florets are best observed when the capitulum is examined on the plant in a green-house during warm, sunny weather. Anthesis begins about 8.30 a.m. in June; half an hour later the tips of some of the styles emerge from the anther tube and by 10 a.m. practically all the florets in the active whorl have their styles completely exerted; each style takes from five to ten minutes to emerge under the best conditions, and they are then sensitive to touch, bending from the base of the thickened portion towards the touch. The thickened portion is hairy and covered with pollen. When in a vigorous condition the style recovers its irritability in less than half a minute, but if touched so that it should react in an opposite direction from the previous movement two or three minutes rest are required in order to get a movement. This male stage of anthesis lasts for only one day in the case of the outermost three rows of hermaphrodite florets. The florets nearer the centre are male, and in these the style acts only as a pollen presenter, and may be withdrawn and exerted again in an irritable condition for two or even three successive evenings and mornings. The styles in these florets are not re-exserted after the male stage, but those of the outer rows of florets are exerted in the female condition with the short style branches spread apart on the second day of anthesis and remain exerted until the conclusion of anthesis. As a rule only one row of styles is in the irritable phase each day at the beginning of the anthesis of the capitulum and two or three in the later stages; there is a diurnal progression of maturity towards the centre of the capitulum.

Arctotis stoechadifolia, Berg.

This species (syn. *A. grandis*) shows a similar series of events, but the stamens are also irritable, so that by touching the anther tube when the style is first exerted one can observe a movement in one direction, while if the style is touched on the opposite side it moves in the opposite direction, or the style may be stimulated first and then the anther tube.

Gazania splendens, X.

This garden hybrid shows the same phenomenon as *A. stoechadifolia* but much more markedly. If the anther tube is touched gently after anthesis has begun the style is exerted with striking rapidity, being fully exerted (to the extent of about half an inch) within two minutes in favourable conditions of light and heat.

With the same touch the anther tube is stimulated to bend towards the touch, and this irritability remains during anthesis. The style is also irritable, and with the first touch after exertion moves as in *Arctotis*, but further touches have little or no effect for at least half an hour unless the same side of the style is touched, when a slight movement in the same direction as the first ensues.

The parents of *Gazania splendens* X are not known, but are said to be two of the three species—*G. ringens*, Gærtn., *G. pavonia*, R.Br. and *G. uniflora*, Sims.

It is noteworthy that all these three genera belong to the Arctotideæ, while three of the genera (*Mimulus*, *Torenia* and *Glossostigma*) in the other families belong to the Gratioleæ of the Scrophulariaceæ, and that these other families themselves all belong to Sections D and E of the Tubifloræ (Engler). The phenomenon seems to be restricted to a few narrow circles of affinities, and is not wide spread as in the case of irritability of the stamens.

C. IRRITABILITY OF THE STAMENS.

The basis of the present section is the following list, Table VIII, (pp. 260-266) of the species on the irritability or non-irritability of which observations have been made. The tribes are arranged according to their origin as shown by the structure of the pollen-presentation mechanism, and the sub-tribes are those of Bentham revised as before. The recognised affinities of the genera are indicated roughly by the numbers, which are those of the Genera Plantarum, except in cases where Bentham sunk a genus which is now upheld, e.g., *Leptosyne*, when a letter is prefixed to the Genera Plantarum number of the containing genus.

Previous authors have not as a rule recorded the exact type of irritability, and those species not observed in the present investigation are indicated thus:—

(Cov) = irritability of the stamens recorded by Covolo;

(K) = " " " " Koelreuter;

(Kb) = " " " " Kabsch;

(Kn) = " " " " Knuth;

(H) = " " " " Haberlandt;

(M) = " " " " Meehan;

(L) = " " " " Linsbauer;

(U) = " " " " Unger;

(J) = " " " " Juel;

(OJ) = absence of irritability recorded by Juel.

The majority of the species have been examined by the writer and the types of irritability are distinguished thus :—

Types of irritability :

A : pollen presented on touching; no lateral movement of the anther tube,

B : pollen presented on touching; lateral movement indefinite in direction,

C : pollen presented on touching; lateral movement towards the touch,

C' : pollen presented on touching; lateral movement towards the centre of the capitulum,

C^e : pollen presented on touching with more or less explosive rapidity,

O : no irritability observed when touched.

The degree of irritability is noted as slight when it requires careful observation, and various other degrees are noted. Various precautions are necessary for the successful observation of the phenomenon, for it was not without reason that Camerarius remarked of the observation of the movement that “*Verum enimvero patientia.*” The general method used in the present investigation was to examine the flowers on a hot, sunny day just after anthesis had commenced, and before an insect had visited the floret, by touching the filaments or anther tube gently with the point of a needle, and by observing the effect with the aid of a watchmaker's eyeglass. The latter was found very convenient as it left both hands free for manipulation. For the details of the precautions readers are referred to the previous paper (49).

The list includes 360 species and varieties, 253 or 70% of which show irritability; all the tribes in the family are represented and 45 of the 64 subtribes, so that the list is sufficiently representative to form the basis of some phylogenetic suggestions.

NOTES ON TABLE VIII.

1. In all these cases (*Tussilagininae*) the critical stage, with the anther tube open and the style not completely exerted, is very short.

2. In *Doronicum Pardalianches* the style has frequently been observed to be bent by pressure against the top of the anther tube

3. *Cineraria Moorei* is a garden hybrid between *C. Héritieri* and a form of *C. cruentes.*

4. *Cineraria stellata* is a florists' variety obtained from Messrs. Dobbie and Sons.

TABLE VIII.

Tribe and Sub-tribe.	Genus and Species.	Type of Irritability.	Remarks.
SENECIONEÆ.			
2 Tussilagininzæ	559 Tussilago Farfara, L.	O	(1)
	560 Petasites albus, Gaert.	O	(1)
3 Senecioninzæ	„ officinalis, Moench.	O	(1)
	564 Arnica alpina, Olin & Ladau.	B	distinct
	„ Chamissonis, Less.	O	
	„ latifolia, Bongard.	O	
	„ longifolia, Eaton.	O	
	565 Doronicum Pardalianches, L.	C	distinct (2)
	„ plantagineum, L.	C	slow
	581 Gynura aurantiaca, D.C.	O	
	582 Cineraria Moorei, (Hort.)	O	(3)
	„ stellata, (Hort.)	A	slight (4)
	583 Emilia sonchifolia, D.C.	O	
	585 Senecio adonidifolius, Loisel.	C	distinct
	„ alpestris, D.C.	O	
	„ aquaticus, Hill.	A: B	A distinct, B slight and slow distinct
	„ bellidioides, Hk.f.	C	
	„ Blumeri, Greene.	O	
	„ clivorum, Maxim.	O	(5)
	„ erucifolius, L.	O	
	„ grandifolius, Less.	O	(6)
	„ Grisebachii, Baker.	O	
	„ hortensis, (Hort.)	C	clear (7)
	„ Jacobaea, L.	C	A distinct, C slight
	„ japonica, Sch.Bip.	C	slight
	„ Ledebouri, Sch.Bip.	A	slight
	„ Ligularia, Hk.f.	A	very slight
	„ „ var. speciosa.	C	very slow
	„ palustris, Hook.	C	distinct
	„ populifolius, D.C.	C	marked (8)
	„ pulvinatus, (Hort.)	A	slight
	„ sarracenicus, L.	(J)	
	„ squalidus, L.	C	distinct
	„ suaveolens, Ell.	(J)	
	„ tropaeolifolius, MacOwan	C	distinct
	„ turkestanicus, C.Winkl.	O	
	„ viscosus, L.	A: C	A distinct, C slight but variable
	„ vulgaris, L.	C	distinct (9)
4 Othonninzæ	589 Othonnopsis cheirifolia, Benth. and Hook.	A	slight
	592 Euryops pectinatus, Cass.	O	
	595 Othonna carnosa, Less.	C	distinct
CICHORIEÆ.			
1 Scolyminzæ	711 Scolymus hispanicus, L.	(K)	
3 Hyoseridinzæ	714 Catananche cærulea, L.	C	very marked (10)
	„ „ var. alba	C	
	717 Cichorium Endivia, L.	C	slow (10)
	„ Intybus, L.	C	distinct (10)
	723 Tolpis barbata, Gaert.	(OJ)	
4 Lapsaninzæ	725 Lapsana communis, L.	C	marked (11)
6 Crepidinzæ	735 Crepis biennis, L.	O	
	„ blattaroides, Vill.	A	distinct (10)
	„ virens, L.	C	distinct (10)
7 Hieraciinzæ	738 Hieracium balkanum, Uechtr.	O	

TABLE VIII (continued)

Tribe and Sub-tribe.	Genus and Species.	Types of Irritability.	Remarks.
7 Hieraciinæ (ctd)	738 Hieracium Bornmuelleri, Freyn.	O	
	„ bupleuroides, Gmel.	O	
	„ Gouani, Hegetsch.	O	
	„ grandifolium, Sch. Bip.	C	slight and slow
	„ maculatum, Schrank.	C	
	„ murorum, L.	C	slight (10)
	„ pallidum, Biv.	C	very slight (10)
	„ Pilosella, L.	O	
	„ prenanthoides, L.	O	
	„ rubrum, Peter.	O	
	„ sabaudum, L.	(K)	
	„ tridentatum, Fries.	O	
8 Hypochæridinæ	742 Hypochæris radicata, L.	C	marked (10)
	„ „ var. hispida, Peterman	C	„ „
	743 Leontodon asperimus, Boiss.	C	distinct (10)
	„ hirtus, L.	C	„ „
	„ hispidus, L.	O	
9 Lactucinæ	745 Taraxacum officinale, Weber.	C	distinct (12)
	750 Lactuca Bourgæi, (=Mulgedium Bourgæi, Boiss.)	C	distinct (10)
	750 Lactuca bracteata, Hk.f.	C	very clear
	„ hastata, DC.	C	distinct
	„ perennis, L.	C	slight
	„ Plumieri, Gren. & Godr.	C	distinct
	„ virosa, L.	C	„
	755 Sonchus arvensis, L.	O	
	„ asper, Hill	C	distinct (10)
	„ oleraceus, L.	C	slight but distinct
10 Scorzonerinæ	760 Tragopogon orientalis, L.	C	distinct
	761 Urospermum Dalechampii, F. W. Schmidt	O	
	762 Scorzonera hispanica, L.	C	very slow but very distinct
	„ purpurea, L.	C	distinct
CALENDULEÆ	597 Dimorphotheca aurantiaca, DC	C	very clear
	„ Ecklonis, DC.	C	marked (13)
	„ pluvialis, Moench.	C	distinct
	598 Calendula arvensis, L.	O	
	„ officinalis, L.	A	slight (14)
ARCTOTIDEÆ			
Arctotidinæ	604 Ursinia anthemoides, Gaert.	(J)	
	„ cakilefolia, DC.	A	distinct
	„ pulchra, N.E.Br.	C	variable (15)
	„ speciosa, DC.	B	„ „
	609 Cryptostemma calendulacea, R.Br.	(OJ)	
2 Gorteriinæ	610 Arctotis stoechadifolia, Berg.	C	distinct (16)
	613 Gazania rigens, Moench.	(OJ)	
	„ splendens, X.	C	distinct (16)
	616 Berkleya purpurea, B. & Hk.f.	O	
ANTHEMIDEÆ			
1 Anthemidinæ	515 Anacyclus officinarum, Heyne	C	distinct
	516 Achillea alpina, L.	C	slight
	„ Clavennæ, L.	C	slight
	„ crustata, Schur.	A	marked
	„ grandiflora, Bieb.	C	marked

TABLE VIII (continued)

Tribe and Sub-tribe.	Genus and Species.	Types of Irritability.	Remarks.
ANTHEMIDEÆ(ctl)			
1 Anthemidinæ (continued)	516 Achillea holosericea, Sibth. and Sm.	A	slight
	„ macedonica, Rouy.	O	(17)
	„ magna, L.	C	distinct
	„ Millefolium, L.	C	marked
	„ nobilis, L.	O	
	„ obscura X, Nees.	O	
	„ Ptarmica, L.	C	distinct (18)
	517 Santolina Chamæcyparissus, L.	C	distinct
	519 Anthemis canescens, Brot.	A	distinct
	„ montana, L., var. grandiflora	C	distinct
	„ nobilis, L.	C	slight
	„ tinctoria, L.	C	distinct
2 Chrysanthem- idinæ.	529 Chrysanthemum atratum, L.	O	
	„ carinatum, Schousb.	C	distinct
	„ coccineum, Willd.	C	distinct
	(=Pyrethrum roseum, Bieb.)		
	„ coronarium, L.	C	distinct
	„ corymbosum, L.	C	slight (19)
	„ ircuitiana, Turcz.	O	
	„ Leucanthemum, L.	C	slight but variable
	„ macrophyllum, Waldst. and Kit.	C	slight
	„ maximum, Raymond	C	slow but distinct
	„ præaltum, Vent.	C	distinct
	„ segetum, L.	A : B	A distinct, B slight
	530 Matricaria Chamomilla, L.	A : C	A distinct, C slight
	„ inodora, L.	A : C	A marked, C distinct
	„ discoidea, DC.	O	
	533 Cotula coronopifolia, L.	O	
	534 Cenia turbinata, Pers.	A : C	A marked, C slight
	548 Tanacetum vulgare, L.	A	distinct
	551 Artemisia vulgaris, L.	(OJ)	
INULEÆ.			
2 Plucheinæ	173 Pluchea Bulleyana, Jeffrey	C	distinct
4 Gnaphaliinæ	203 Antennaria dioica, Gaert.	(H)	(20)
	208 Anaphalis cinnamomea, C.B.C.	C	distinct
	„ margaritacea, Benth.	C	distinct
	219 Helipterum Manglesii, Muell.	C	marked (21)
	„ roseum, Benth.	A	distinct (14)
	220 Helichrysum bracteatum, Andr.	C	slight
	225 Cassinia Vauvilliersii, Hk.f.	A	distinct
	235 Ammobium alatum, R.Br.	A	slight
5 Angianthinæ	244 Myriocephalus Stuartii, Benth.	C	short, quick movement
7 Athrixiinæ	269 Podolepis canescens, A.Cunn.	(OJ)	
8 Inulinæ	275 Inula Conyza, DC.	C	slight
	„ ensifolia, L.	O	
	„ glandulosa, Puschk.	C	distinct
	„ Helenium, L.	(J)	
	289 Pulicaria vulgaris, Gaert.	A	distinct
	„ dysenterica, Gray.	A : C	A distinct, C variable

TABLE VIII (continued)

Tribe and Sub-tribe.	Genus and Species.	Types of Irritability.	Remarks.
INULEÆ (ctd.)			
9 Bupthalmiæ	298 Bupthalmum salicifolium, L.	B	distinct
	" speciosum, Schreb.	C	slow
	302 Odontospermum maritimum, Sch.Bip.	C	marked (22)
CYNAREÆ			
1 Echinopsidinæ	621 Echinops bannaticus, Rochel.	C	slight
	" cornigerus, DC.	B	distinct
	" dahuricus, Fisch.	C	distinct
	" exaltatus, Schrad.	C	marked
	" Ritro, L.	C	distinct (23)
	" sphærocephalus, L.	C	marked
	" Tourneforti, Ledeb.	C	marked
2 Carliniæ	624 Xeranthemum annuum, L.	(J)	
3 Carduinæ	631 Arctium majus, Bernh.	C	slight
	633 Carduus cernuus, Steud.	C	marked
	" crispus, L.	C	distinct
	" defloratus, L.	(Kn)	
	" nutans, L.	C	distinct
	" Personata, Jacq.	(Kn)	
	" tenuiflorus, Curt.	B	distinct
	634 Cnicus acaulis, Willd.	C	distinct
	" altissimus, Willd.	(M)	
	" arvensis, Hoffm.	(J)	
	" canus, Roth.	(J)	
	" carlinoides, Fisch.	(H)	
	" Casabonæ, Roth.	(K)	
	" eriphorus, Roth.	B	slight
	" lanceolatus, Willd.	C	distinct
	" palustris, Willd.	C	marked (24)
	" serrulatus, Bieb.	(M)	
	" syriacus, Roth.	(J)	
	634a Cirsium Kernerii X (Hort.)	B	distinct
	635 Onopordon acanthium, L.	(K)	
	" arabicum, L.	(K)	
	636 Cynara Cardunculus, L.	(K)	
	637 Silybum Marianum, Gaert.	C	distinct
	639 Tyrimnus leucographus, Cass.	(J)	
	642 Saussurea albescens, Hk.f.	C	marked
	" Yakla, G.B.C.	C	distinct (25)
4 Centaureinæ	648 Serratula coronata, L.	(J)	
	" quinquefolia, Bieb.	C	slight, variable
	653 Leuzea conifera, DC.	C	distinct
	654 Centaurea alba, L.	(M)	
	" americana, Nutt.	(M)	
	" aspera, L.	B	distinct
	" atropurpurea, Waldst. and Kit.	C	slight
	" axillaris, Willd.	C'	distinct
	" calcitrapoides, L.	(Cov.)	
	" cineraria, L.	(K)	
	" concolor	(U)	
	" Cyanus, L.	C'	distinct
	" dealbata, Willd.	C	distinct
	" eriphora, L.	(K)	
	" glastifolia, L.	(K)	
	" Jacea, L.	(J)	
	" macrocephala, Puschk.	(Kb)	

TABLE VIII (continued)

Tribe and Sub-tribe.	Genus and Species.	Types of Irritability.	Remarks.
CYNAREÆ (ctd.)			
4 Centaureinæ (continued)	654 <i>Centaurea maculosa</i> , Lam.	(L)	
	„ <i>montana</i> , L.	C	distinct
	„ „ var. <i>alba</i>	C	distinct
	„ „ var. <i>lugdunensis</i> , Jord.	C	distinct
	„ <i>Moschata</i> , L.	(K)	
	„ <i>nervosa</i> , Willd.	(Kn)	
	„ <i>nigra</i> , L.	C	variable (26)
	„ „ var. <i>alba</i>	C	marked
	„ <i>orientalis</i> , L.	(H)	
	„ <i>præalta</i> , Boiss. & Bal.	C	distinct
	„ <i>pulchella</i> , Ledeb.	(Kb)	
	„ <i>pulcherrima</i> , Willd.	O	(27)
	„ <i>ragusina</i> , L.	(K)	
	„ <i>ruthenica</i> , Lam.	(Kb)	
	„ <i>rulifolia</i> , Sibth.	(M)	
	„ <i>salmantica</i> , L.	(K)	
	„ <i>Scabiosa</i> , L.	C	distinct
	„ <i>sordida</i> , Willd.	B	marked
	„ <i>spinosa</i> , L.	(K)	
	„ <i>variegata</i> , Lam.	C	distinct
	655 <i>Carbenia benedicta</i> , Adans.	(K & J)	
	656 <i>Carthamus tinctorius</i> , L.	C	distinct
MUTISIEÆ.			
4 Gerberinæ	695 <i>Gerbera hybrida</i> , (Hort.)	C*	(28)
	„ <i>Jamesoni</i> , Bolus	C*	(28)
5 Nassauviinæ	702 <i>Perezia multiflora</i> , Less.	C*	(28)
	708 <i>Moscharia pinnatifida</i> , Ruiz and Pav.	A : B	A distinct, B variable
VERNONIEÆ.			
1 Vernoniinæ	6 <i>Erlangea tomentosa</i> , S. Moore	O	
	16 <i>Vernonia altissima</i> , Nutt.	O	
	„ <i>fasciculata</i> , Michx.	O	
	28 <i>Stokesia cyanea</i> , L'Hérit.	O	
ASTEREÆ.			
1 Homochrominæ	79 <i>Grindelia cuneifolia</i> , Nutt.	O	
	„ <i>lanceolata</i> , Nutt.	O	
	„ <i>squarrosa</i> , Dimal.	C	distinct
	85 <i>Chrysopsis vilosa</i> , DC.	C	variable
	„ „ var. <i>Rutteri</i>	O	
	88a <i>Aplopappus croceus</i> , Gray	C	distinct
	91 <i>Solidago serotina</i> , Ait.	C	distinct
	„ <i>Virgaurea</i> , L.	C	very slight
	„ <i>piloso</i> , Walt.	C	distinct
3 Bellidinæ	116 <i>Brachycome iberidifolia</i> , Benth	O	
	„ <i>Thomsoni</i> , Kirk.	O	
	117 <i>Bellis perennis</i> , L.	C	variable (29)
4 Heterochrominæ	119 <i>Amellus annuus</i> , Willd.	C	slight
	121 <i>Charieis heterophylla</i> , Cass.	C	distinct
	134 <i>Callistephus hortensis</i> , Cass.	(J)	
	136 <i>Aster acris</i> , L.	C	distinct
	„ <i>alpinus</i> , L.	O	
	„ <i>Amellus</i> , L.	O	
	„ <i>Candollii</i> , Harv.	A	slight
	„ <i>Curtisii</i> , Torr. & Gray	A : C	A distinct, C slight
	„ <i>Lipskyi</i> , (Hort.)	C'	marked

Origin and Development of the Compositæ. 265

TABLE VIII (continued)

Tribe and Sub-tribe.	Genus and Species.	Types of Irritability.	Remarks.
ASTEREÆ (ctd.)			
4 Heterochrominæ (continued)	136 <i>Aster longifolius</i> , Lam.	C	variable
	„ <i>macrophyllus</i> , L.	(J)	
	„ <i>oreophilus</i> , Franch.	O	
	„ <i>paniculatus</i> , Lam.	A	slight
	„ <i>puniceus</i> , L.	A : B	A distinct, B slight
	„ <i>scaber</i> , Thunb.	O	
	„ <i>sibiricus</i> , L.	O	
	„ <i>stellaris</i> , (Hort.)	C	variable
	„ <i>trinervius</i> , Roxb.	O	
	„ <i>Tripolium</i> , L.	O	
	137 <i>Felicia fragilis</i> , Cass.	C	distinct
	142 <i>Olearia dentata</i> , Moench.	A	very slight
	„ <i>Haastii</i> , Hook.	O	
	147 <i>Celmisia coriacea</i> , Raoul.	C	distinct
	„ <i>petiolata</i> , Hook.	O	
	„ <i>verbascifolia</i> , Hook.	O	(30)
	151 <i>Erigeron aurantiacus</i> , Regel.	A	distinct
	„ <i>Coulteri</i> , Porter	A	distinct (31)
	„ <i>glabellus</i> , Nutt.	A	distinct
	„ <i>grandiflorus</i> , Hook.	A	distinct
	„ <i>macranthus</i> , Nutt.	C	distinct
	„ <i>multiradiatus</i> , Benth. and Hook.	C	distinct (32)
	„ <i>Rusbyi</i> , Gray	A : B	A distinct, B slight
	„ <i>speciosus</i> , DC.	A	distinct
5 Conyzinæ	162 <i>Chrysocoma Coma-aurea</i> , L.	C	distinct
EUPATORIÆÆ.			
2 Ageratinæ	54 <i>Ageratum conyzoides</i> , L.	(OJ)	
	66 <i>Eupatorium ageratoides</i> , L.	O	
	„ <i>cannabinum</i> , L.	O	
	„ <i>ianthinum</i> , Hemsl.	O	
	„ <i>perfoliatum</i> , L.	O	
	„ <i>prunellæfolium</i> , H.B. & K.	O	
	„ <i>purpureum</i> , L.	O	
	„ <i>riparium</i> , Regel.	O	
3 Adenostylinæ	73 <i>Liatris callilepsis</i> , (Hort.)	O	
	„ <i>spicata</i> , Willd.	O	
HELENIÆÆ.			
2 Baeriinæ	457 <i>Baeria coronaria</i> , Gray	O	
	460 <i>Lasthenia glabrata</i> , Lindl.	O	
	468a <i>Eriophyllum cæspitosum</i> , Dougl.	O	
	476 <i>Palafoxia Hookeriana</i> , Torr. and Gray	(J ?)	
4 Tagetinæ	496 <i>Tagetes erecta</i> , L.	(OJ)	
	„ <i>patula</i> , L.	(OJ)	
	„ <i>signata</i> , Bartl.	(OJ)	
5 Heleniinæ	501 <i>Helenium autumnale</i> , L.	C	distinct
	„ „ <i>var. bicolor</i>	C	distinct
	„ „ <i>var. pumilum</i>	C	slight
	„ <i>Hoopesii</i> , Gray	C	distinct
	502 <i>Gaillardia aristata</i> , Pursh.	(OJ)	(33)
	„ „ <i>var. grandiflora</i>	C	distinct (33)
	„ <i>lanceolata</i> , Michx.	O	

TABLE VIII (continued)

Tribe and Sub-tribe.	Genus and Species.	Types of Irritability.	Remarks.
HELIANTHIEÆ			
3 Melampodiinæ	333 <i>Silphium Astericus</i> , L.	O	
	„ <i>integrifolium</i> , Michx.	A	slight
	„ <i>perfoliatum</i> , L.	O	
	334 <i>Chrysogonum virginianum</i> , L.	O	
4 Ambrosiinæ	336 <i>Lindheimera texana</i> , Gray	A	marked
	338 <i>Parthenium integrifolium</i> , L.	O	
	347 <i>Ambrosia artemisiifolia</i> , L.	(OJ)	
	349 <i>Xanthium spinosum</i> , L.	(OJ)	
6 Zinniinæ	„ <i>Strumarium</i> , L.	(OJ)	
	355 <i>Zinnia elegans</i> , Jacq.	C	distinct
	„ <i>Haageana</i> , Regel.	(OJ)	
	356 <i>Sanvitalia procumbens</i> , Lam.	(J)	
7 Verbesininæ	357 <i>Heliopsis lævis</i> , Pers.	C	distinct
	„ <i>padula</i> , Wender.	C	slow but distinct
	„ <i>scabra</i> , Dun.	C	distinct (34)
	„ „ <i>var. gratissima</i> , Lemoin.	C	distinct (35)
	„ „ <i>var. pitcheriana</i>	C	very slow but distinct
	379 <i>Rudbeckia ampla</i> , Nelson	C	distinct
	„ <i>amplexicaulis</i> , Vahl.	(J)	
	„ <i>californica</i> , Gray	O	
	„ <i>hirta</i> , L.	C	see Halsted, 18
	„ <i>nitida</i> , Nutt.	C	distinct
	„ <i>speciosa</i> , Wender.	C	marked
	„ <i>triloba</i> , L.	C	slight
	379a <i>Echinacea angustifolia</i> , DC.	C	see Halsted, 18
	„ <i>purpurea</i> , Moench.	C	distinct
8 Coreopsidinæ	379b <i>Lepachys pinnatifida</i> , Rafn.	C	see Halsted, 18
	397 <i>Wyethia mollis</i> , Gray	O	
	400 <i>Helianthus annuus</i> , L.	C	slight (36)
	„ <i>debilis</i> , Nutt.	(OJ)	
	„ <i>hirsutus</i> , Rafn.	(M)	
	„ <i>lenticularis</i> , Dougl.	(M)	
	„ <i>micranthus</i> , Spreng.	O	
	„ <i>mollis</i> , Lam.	A	distinct
	„ <i>occidentalis</i> , Ridd.	A : C	A distinct, C slight
	„ <i>pumilus</i> , Nutt.	O	
	„ <i>rigidus</i> , Desf.	A : B	A distinct, B slight
	„ <i>tomentosus</i> , Michx.	O	
	405 <i>Helianthella quinquenervis</i> , Gray	O	
	416 <i>Guizotia abyssinica</i> , Cass.	C	distinct
9 Galinsoginæ	423 <i>Coreopsis Atkinsoniana</i> , Dougl.	C	slight
	„ <i>Drummondii</i> , Torr. & Gray	(OJ)	
	„ <i>grandiflora</i> , Nutt.	A	slight
	„ <i>Grantii</i> , Oliv.	A : C	A distinct, C slight
	„ <i>tinctoria</i> , Nutt.	A	distinct
	423a <i>Leptosyne Douglasii</i> , DC.	O	
	„ <i>maritima</i> , Gray	A	slight
	„ <i>Stillmannii</i> , Gray	O	
	424 <i>Dahlia coccinea</i> , Cav.	O	
	„ <i>Merckii</i> , Lehm.	O	
10 Madiinæ	„ <i>variabilis</i> , Desf.	(OJ)	
	426 <i>Thelesperma filifolium</i> , Gray	O	
	427 <i>Cosmos atosanguineus</i> , (?)	(J)	
	428 <i>Bidens grandiflora</i> , Balb.	A	slight
9 Galinsoginæ	435 <i>Balduinia multiflora</i> , Nutt.	C	slight but distinct
	438 <i>Tridax trilobata</i> , Hemsl.	A	slight
10 Madiinæ	442 <i>Madia dissitiflora</i> , Torr. & Gray	O	
	„ <i>sativa</i> Molin.	O	
	445 <i>Layia gaillardoides</i> , Hook & Arn	C	very slight

5. In *Senecio clivorum* the filaments are quite unusually thick and rigid.

6. In *Senecio grandifolius* the male stage is very short and the anther tube is frequently split.

7. *Senecio hortensis* seems to be a variety of *S. populifolius*.

8. In *Senecio populifolius* when the anther tube is touched at the proper stage the style protrudes quickly as in *Gazania*, and the style branches may be seen to diverge immediately the style is exerted and the anthers are retracted sufficiently.

9. The movement is exceptionally difficult to observe in *Senecio vulgaris* as the proper stage is rare on any one plant, and the movement is distinct only during hot weather.

10. The lateral movement in the Cichorieæ is slow, and the retraction of the anther tube requires careful observation. It frequently continues after the style branches have diverged.

11. In *Lapsana communis* the straightening of a single filament when touched can be observed easily; the other filaments are seen to become more bent, thus tilting the anther tube in the direction of the stimulated filament.

12. The critical stage in *Taraxacum officinale* is short, and the movement is shown only when the exertion of the style is beginning.

13. *Dimorphotheca Ecklonis* has orange pollen and dark purple stamens, the presentation of the pollen is therefore very easily seen.

14. *Calendula officinalis* and *Helipterum roseum* are recorded by Juel (28) as not irritable, but it must be borne in mind that these show the "A" type only, and that species which are irritable in these latitudes may not show the character further north, cp. *Torenia* (Section B, Chap. III).

15. *Ursinia pulchra* is very variable, showing "A" distinctly under normal conditions in the north (Newcastle), "B" on warm days and "C" under exceptionally favourable circumstances. *U. speciosa* is also very variable, but the "C" type has not yet been observed in this species.

16. For details of the double movement in the stamens and the style in *Arctotis stoechadifolia* and *Gazania splendens* see Section B, Chap. III).

17. The anther tube in *Achillea macedonica* is frequently split by the style.

18. *Achillea Ptarmica* usually shows "A" distinctly. At 10 a.m. in the north (Forfarshire) it shows "C" slightly, but by noon it shows "C" distinctly.

19. *Chrysanthemum corymbosum* shows no movement until the critical stage when the anther tube is opening, and the style is frequently bent as in *Doronicum*.

20. The irritability in *Antennaria dioica* is described by Hildebrand (23) as similar to that of the *Cynareæ*, so that it probably shows the "C" type.

21. In *Helipterum Manglesii* the movement towards the touch through an angle of about twenty degrees is immediately followed by a movement in the reverse direction of about half that magnitude.

22. In *Odontospermum maritimum* the lateral movement is rapid, and is accompanied by a rapid ejection of the pollen, cp. Mutisieæ, note 28.

23. The movement in *Echinops Ritro* occurs only before the style is exerted, and usually only when the filament itself is touched (see note 24).

24. In *Cnicus palustris* and most other species the lateral movement is greatest when the filament itself is touched, not the anther as is usual.

25. The anther tube in *Saussurea Yakla* projects above the disc just high enough to rub the pollen at the top against the wing of a bee while it is standing on the disc. This mechanism is easily observed while bees are visiting the flower.

26. The variation in the movement in *Centaurea nigra*, sometimes "B" and sometimes "C," is partly explained by the behaviour of the filaments. When the anther tube is touched at one side all the filaments can be seen to become more bent and then to straighten out, at the same time becoming shorter. The two stages seem to give time for one of the filaments other than the nearest one to contract first and the successive contractions give the type "B."

27. This exception (*Centaurea pulcherrima*) in the genus may be explained by the fact that a considerable part of the style is free from hairs and the pollen-presentation begins only after the exertion of the style to a considerable degree.

28. In *Gerbera hybrida* the pollen is extruded in small quantity but more rapidly than is usual in the "C" type: the movement is towards the touch. In *Gerbera Jamesoni* and *Perezia multiflora* the outer florets are much more active than the inner, and the greatest lateral movement is towards the centre of the capitulum. In the former species half the pollen is expelled at the first touch and the rest at the second. In the latter all the pollen

is expelled at the first touch. This explosive movement has as yet been observed only in the Mutisiæ, and is sometimes so energetic that the pollen is thrown over the edge of the capitulum.

29. The movement in *Bellis perennis* is most active, as in many other species, between noon and 3 p.m., and is sometimes so strong that the anther tube is split against the comparatively thick and rigid style.

30. The anther tube is frequently split in *Celmisia verbascifolia*, cp. notes 2, 6 and 17. This character is also recorded for *Cratistylis conocephala* (49, p. 267, note 8).

31. When repeatedly tested with a cloud over the sun *Erigeron Coulteri* showed no movement, but an hour later with a few minutes of sunshine the "A" type of movement was distinct.

32. The movement is greatest in *Erigeron multiradiatus* when touched so that the reaction is towards the centre of the capitulum.

33. Juel (28) records no irritability in *Gaillardia aristata* and the var. *grandiflora* showed none at 6.30 p.m., but was distinctly sensitive at 10.30 a.m. (Kew).

34. The movement is greatest in *Heliopsis scabra* when it is towards the centre, cp. note 32.

35. In var. *gratissima* of the above species the anthers are forced apart when the filament is stimulated and the pollen is presented along the slit. In the evening the stamens are more or less free from each other, cp. notes 2, 6, 17 and 30.

36. The lateral movement in *Helianthus annuus* is slight and slow, but Gray (13) previously recorded a distinct lateral movement (see Section A, Chap. III).

The physiology of the movement is discussed in Section D and the phylogenetic significance in Section E, so that it is only necessary to point out some of the chief facts of the distribution of irritability in the family as shown by Table VIII.

Of the three main types, A, B and C, of movement C seems to be the most primitive because it involves only one step—the contraction of one of the five filaments; B involves the transference of the stimulus to the other four filaments, and this occurs in an indefinite order, giving an indefinite lateral movement; A involves the transference of the stimulus to the other four filaments in such an organised fashion that all five contract simultaneously.

In the cases where the A type of movement is recorded as slight it is possible that this is due to an incipient C motion, which would show as such in better conditions. The A type in the Anthemideæ, *Lindheimera*, *Coreopsis* and *Erigeron*, however, seems

to be a distinct movement of a higher type. C' and C^e are obviously special developments of the general C type.

In *Senecio* the C type is the commonest, while allied genera such as *Arnica*, *Cineraria* and *Othonnopsis* show the higher types, A or B. The Tussilagininæ and Vernoniæ with a pollen presentation like that of the Eupatoriæ show no irritability, nor does the latter tribe. The Cichoriæ are distinguished by a peculiar, slow, lateral movement of the primitive C type; this type of movement also occurs in *Senecio Ligularia*, var. *speciosa*. The C type is very active and highly developed in the Calenduleæ, notably in *Dimorphotheca*. The Arctotideæ combine an irritable style with the C type of staminal movement. The Anthemideæ and Inuleæ show an increase in the type A movement, while type C remains predominant. Six of the nine sub-tribes in the Inuleæ have been examined and five show irritability. Type B is shown in a number of Cynareæ, and this is to be correlated with the occurrence of the same type in *Bupthalmum salicifolium*. Type C' seems to be confined to the genus *Centaurea*, except for the intermediate stage shown by *Erigeron* and *Heliopsis* (see notes 32 and 34). The explosive type, C^e, is confined to the Mutisieæ.

Five of the six sub-tribes in the Astereæ have been examined and all show irritability, mostly of the C type, but with type A common and developed to a notable extent in *Aster* and even more so in *Erigeron*. Three of the five tribes in the Heleniæ have been examined, and only one (Heleniæ) shows irritability with certainty. This is noteworthy, and is to be correlated with the number of genera in the Heliantheæ which show no irritability. Seven of the ten sub-tribes in the Heliantheæ have been examined, and six show irritability. Irritability is scarcely to be expected in the dioecious forms of the Ambrosiinæ; the predominance of the C type in the Verbesiinæ and the large proportion of the A type in the Melampodiinæ and Coreopsidinæ are to be noted.

D. PHYSIOLOGY OF IRRITABILITY.

Apart from such statements as that of Bose (3) that the stamens in the Compositæ are truly sensitive, Cassini's suggestion (6) of the death of the filaments as the cause, that of Ker (30) that the movement of the style in *Arctotis* is due merely to elasticity and Meehan's similar explanation (41) of the movement of the stamens in *Helianthus*, with the contradictions by Brown (4) and Gray (13-14), considerable progress has been made with the study of the general phenomena of irritability. Smith (51)

discovered the localisation of the irritability in *Berberis* and Goeppert (12) and others followed with physiological studies of that genus. Kabsch (29) seems to have been the first to make a proper physiological investigation of the movement in *Centaurea*. He figures and describes the vascular supply and the tactile hairs. He suggests that the spiral vessels are perhaps responsible for the well-known elasticity of the filaments, and considers that the tactile hairs occur so frequently in all irritable floral organs that they appear to be the chief factor in irritability.

Heckel (22) distinguishes between nutritive irritability with an automatic mechanism as in *Ruta* and functional irritability as in the many other cases where a touch is required to set the mechanism in motion. The latter class can be conveniently sub-divided into those cases where the action is mechanical, as in the explosive stamens of *Urtica*, and those where the response is protoplasmic in origin, as in *Berberis* and the Compositæ.

Pfeffer (45) made the next important contribution, and there is an easily accessible account of this work in his *Physiology of Plants* (46). He shows that in *Berberis* the mechanism is similar to that of the pulvinus in *Mimosa*, and that the structure and distribution of the irritable tissue in *Helianthemum*, etc., cause the stamens to move always in the same direction wherever they are touched. He also shows that the contraction of the filaments in the *Cynaræ* is due to a loss of turgor, that the filaments contract with a decrease in volume and the exudation of water into the inter-cellular spaces, which are largely developed in the tissue of the filament. The contraction varies from 8% to 30% of the length of the stamens, and as the injection of a .5—1.0% solution of potassium nitrate causes the same amount of contraction Pfeffer concluded that the energy of contraction amounts to from 1 to 3 atmospheres pressure. On these grounds he controverts Cohn (9) and Unger (54-55), who maintained that the filaments broadened as they contracted and that there was no diminution in volume. Cohn also considered that the active contraction of the protoplasm was responsible for the movement, but Pfeffer considered this improbable on account of the high energy of contraction. He also contradicts Hofmeister (24), who suggested that the cell-wall was the responsive part of the cell, although he agrees that the power of contraction lies in the cell-wall, and shows that the filaments after stimulation and contraction when put in boiling water contract 10%—40% of their length more on account of the elasticity of the cell-wall.

Oliver (44) showed that in the case of the stigmas of *Mimulus*

and *Martynia* the transmission of the stimulus was completely independent of the vascular bundle, and that the presence of a well developed system of intercellular spaces confirmed Pfeffer's theory of the extrusion of water and loss of turgor as the cause of the movement. Chauveaud (8) controverts Pfeffer and returns to a position similar to that of Cohn. Minden (42), however, considers that the style of *Arctotis* shows analogies in structure and movement with those described by Pfeffer in the stamens of the *Cynareæ*. The marked effect of the meteorological conditions on the movement of the styles (47) and stamens (49) is another piece of confirmatory evidence. The fact recorded by Harger (21), that if no pollen is placed between the stigmatic lobes of *Martynia* they soon re-open but with pollen this never occurs, also shows that the movement is no mere mechanical one.

Most of those who have described the tactile hairs, especially Haberlandt (15), consider them to be organs of perception, but Halsted (18) has recorded a movement in at least two species in which no tactile hairs are present. Many of the species given in Table VIII also show no tactile hairs and Linsbauer (37) regards these hairs when present as stimulators or stimulus-transmitters rather than organs of perception.

It may be considered proved that a loss of turgor when the filament or style is touched is the cause of the movement, and Haberlandt states that "it is not known whether the fall of turgor depends upon a sudden diminution of the osmotic strength of the cell-sap, caused by the external stimulus, or whether the latter brings about an increase of the permeability of the ectoplast towards the osmotically effective constituents of the cell." If we consider that the cell-sap has a much more stable composition than the ectoplast, and that the latter is protoplasmic and therefore in a condition of unstable equilibrium, it is not difficult to see which is the more likely to be affected by a sudden change on being touched. Dr. F. F. Blackman in a recent lecture (London, 1913) suggested that the irritability of the stamens in the *Cynareæ* might be due to permeability changes similar to those in the pulvini of *Mimosa* and *Phaseolus*. The writer (50) has recently shown that the geotropic sensitiveness of the root is due to permeability changes giving a differential loss of turgor and consequent movement. The difference in the time relations of the movement in the root and the filaments is to be explained by the state of tension of the latter and their relative slimmness. In those cases where tactile hairs are absent the same deformation of the sensitive protoplasm may occur in the epidermal cells as probably

occurs in the tactile hairs when they are touched, thus starting the changes which result in the movement.

It is hoped that the necessary apparatus will be devised presently to prove the similarity, if it exists, in the stimulation of the root and of the stamens, and it may be possible also to test a suggestion that in both cases the first result of the application of the stimulus is a change in the electrical condition of the limiting layer of the protoplasm, which change alters the permeability, and therefore the turgor, giving the elastic cell-walls an opportunity to contract in the case of the filaments and to expand at a relatively greater rate on the upper side in the case of the root.

E. THE PHYLOGENETIC SIGNIFICANCE OF IRRITABILITY

Considering all these facts in the light of the probable lines of evolution (found as a result of the examination of the morphology of the styles and stamens) shown in Fig. 7, Chap. II, the first point to be noticed is that in no case is a sub-tribe which shows irritability derived from one showing no irritability. This fact in itself is very good proof that irritability has followed the same lines of evolution as the morphology of the pollen-presentation mechanism. The second point is that the possible affinities of the Gnaphaliinæ with the Gorteriinæ and Gerberinæ are rendered less probable by the occurrence both in the Gorteriinæ and Arctotidinæ of irritable styles of the same type, and by the occurrence in both the Nassauviinæ and Gerberinæ of an explosive mechanism of a peculiar type. These two pairs of sub-tribes seem to be more intimately connected than is suggested by the morphology of their styles and stamens. The development of the A type in several distantly related groups is not of great importance, as that type of movement is a logical development in the evolution of irritability.

The Cichorieæ are again isolated on account of the peculiar, slow movement of the anther tube, which movement, however, is also shown by *Senecio Ligularia*, var. *speciosa*.

The Tussilaginæ, Vernoniæ, Eupatorieæ, Ambrosiinæ and Athrixiinæ are all end groups derived from groups in which irritability is present, so that the absence (as yet) of records of irritability for these groups does not in any way invalidate the previous arguments. As before the Othonninæ cannot be distinguished from the Senecioninæ, nor can the various tribes of the Cichorieæ be separated from each other with any certainty.

The Calenduleæ show a marked development of the C type in *Dimorphotheca*, thus confirming the affinity with the Senecioninæ.

The Arctotideæ are again isolated on account of their irritable

styles, but by the same phenomenon the tribe is shown to be more homogeneous. The occurrence of types A and B, as well as C, but no irritable style in *Ursinia* is very interesting as that genus was suggested (Chap. II, E) as the primitive genus in the tribe.

The two sub-tribes in the Anthemideæ again cannot be distinguished.

The large development of irritability (90% or 18 out of 20 species) in the Inuleæ is quite in accordance with the suggested derivation of the Cynareæ from that tribe, and the marked movement in *Odontospermum* and the occurrence of the B type in *Buphthalmum* also support the same suggestion. Only one of the Cynareæ examined (*Centaurea pulcherrima*) showed no irritability, but the pollen-presentation is somewhat anomalous, and further investigation is required. Irritability is highly developed in this tribe, and the type A movement is combined in a marked degree with the lateral movement of the C type, so that the advanced position of the Cynareæ is maintained.

In the Mutisieæ it was suggested that the Nassauviinæ was the primitive group, and this is supported by the occurrence of the A and B types in *Moschardia* of that sub-tribe and the development of the explosive mechanism in *Perezia* and the Gerberinæ.

In the Astereæ the absence of the A type from the Homochrominæ and the Conyzinæ may be taken as the forerunner of the complete absence (by disappearance) of irritability in the Eupatorieæ, or it may be that the Heterochrominæ with type A is the higher group. The development of the A type in the Heterochrominæ confirms that group as one in advance of the Senecioninæ, while the absence of the A type in the Bellidinæ is to be explained by its relative nearness to the primitive stock.

The Heleniæ are particularly interesting. On account of their comparatively advanced stamens (types 4-6) the Heleniinæ were placed above the Tagetinæ (type 3), but were derived from the stock before the latter on account of the simplicity of their styles (type IV) as compared with the Tagetinæ (types VII-VIII). Now, while the latter shows no irritability, the Heleniinæ do so to a large extent. The other sub-tribes were derived from the Tagetinæ, and show no irritability.

In the Heliantheæ the A type movement, which is undoubtedly distinct from the C type, occurs in the Coreopsidinæ and Melampodiinæ, two sub-tribes which are placed in the same line of evolution in the original phyletic scheme.

We therefore come to the conclusion that the evolution of the sub-tribes suggested in Fig. 7, Chap. II, is confirmed by the

study of the irritability of the pollen-presentation mechanism in every point upon which we can bring it to bear in the present strictly limited condition of our knowledge of the distribution of irritability in the family. There are many gaps which it is hoped to fill by a more extensive study of the phenomenon.

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276 *Origin and Development of the Compositæ.*

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